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PROTEIN NUTRITION

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*This series of papers is the result of a conference on *Protein Nutrition* held by The New York Academy of Sciences on March 1 and 2, 1957. The conference and this monograph were made possible through the cooperation and support of E. I. du Pont de Nemours & Company, Inc., Wilmington, Del.

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THE PROTEIN REQUIREMENTS OF CHILDREN FROM ONE TO TEN YEARS OF AGE*

By Genevieve Stearns,† Katherine J. Newman,‡ J. B. McKinley,†
and P. C. Jeans§

Department of Pediatrics, College of Medicine, State University of Iowa, Iowa City, Iowa

The studies reported here are a part of a 25-year investigation undertaken with the efficient aid of our nursing staff and of assistants and graduate students in our laboratory. The original study was designed to cover the period of growth and to determine the nutritional requirements at each age in relation to and as affected by the preceding period of growth and its nutrition. This discussion concerns the protein requirements of children aged 1 to 10 years.

With but 2 exceptions, our subjects under 2 years of age were children of students or staff or had been in our study ward since infancy. A few 2-year-olds were staff children; other than these, the subjects were loaned to us by orphanages. Boys predominate heavily in the group, because boys predominated in the orphanage populations. The orphanage children were within average range for weight and height, but somewhat below the mean for Iowa City children,[1] a privileged group. Their general status could be listed as lower middle class. All children were housed in a separate ward with its own playground. The nursing staff was chosen primarily because of ability in handling infants and children with "motherly love and patience." School and recreational facilities were provided, and a serious attempt was made to duplicate the daily life and activities of a healthy American child as far as possible.

Children from the orphanages were housed for a month or more before studies were begun, both to accustom them to their new surroundings and to adjust them to a higher protein diet than the average orphanage offered.

Periods of quantitative study for young children were only of 3-day duration, but were numerous; studies of infants had shown that physical restraint for longer than 3 days at a time decreases efficiency of utilization of food. Because of short periods of study, the constancy of collection is less accurate in these children and is compensated for by a larger number of studies.

Older children were usually studied for 2 or 3 consecutive 5-day periods 10 to 15 days after a change in dietary regimen.

The studies were begun in 1930 and were carried on as opportunity offered. Each age was studied in at least 2 nonconsecutive series, so that inadvertent changes in regimen would not color the results. The data reported here include 458 studies of 51 children 1 to 4 years of age, and 481 studies of 67

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* The work reported in this paper was carried out largely during the period of a continuation grant from Mead Johnson & Company, Evansville, Ind. The authors are indebted to the National Institutes of Health, Public Health Service, Bethesda, Md., for a grant covering the expenses of preparing the data for publication.

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children 4 to 11 years of age. Emphasis has been placed on children under 4 years of age because of the paucity of data in the literature for this important age range.

FIGURE 1 shows the mean caloric intake for the children studied. The blocks indicate the 3-year mean values given in the 1953 Recommended Allowances.[2] It appears that the children's mean caloric intake matched the Recommended Allowances very closely, although each child's caloric intake was guided largely by his own appetite. FIGURE 2 shows the mean protein intake, both as estimated from tables[3] and as calculated from nitrogen analyses.

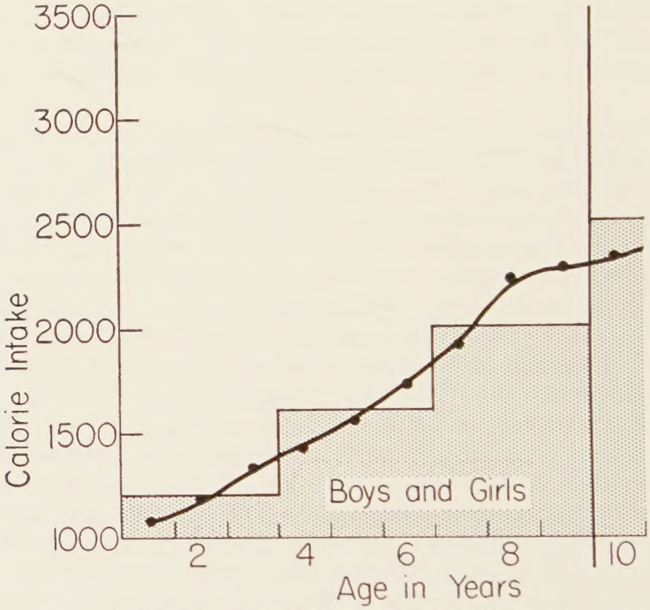

FIGURE 1. Mean caloric intake of the children included in this study as compared to the Recommended Nutritional Allowances for this age range.[2]

The 2 methods of estimation agree well. The mean protein intake of our subjects was well above that presently recommended by the National Research Council, Washington, D. C. Although protein intakes were graded, no child was knowingly put under the stress of a protein intake expected to be below maintenance requirement. On the other hand, no attempt was made to increase the protein intake above levels that the child would willingly ingest from common foods. The data cover the practical range of protein intake common to American children.

FIGURE 3 shows the mean protein intake as a percentage of total caloric intake. The range varied from 16 per cent for 1- and 2-year-olds to 13 per cent for 10-year-old boys, all sharply above Recommended Allowances.

The requirement of a child for any given nutrient may be defined as the intake that will permit the rate of growth and development normal for the child's age. Unfortunately, no consensus exists as to the exact rate of growth considered normal. This rate is defined here as the mean growth of children not restricted in the intake of any essential nutrient and protected against adverse influences of environment. The protein requirement at any age will depend on the resultant of the rate of growth and the nitrogen content of each tissue at that age. These resultants are not necessarily reflected in the rate of growth of the body as a whole. The well-known slowing of growth in both height and weight that begins during the latter part of the first year and continues at a progressively slower rate to 4 or 5 years of age is not accompanied by slowed growth of each tissue. Similarly, the steady growth through mid-childhood may not be accompanied by constant rate of growth of each single tissue.

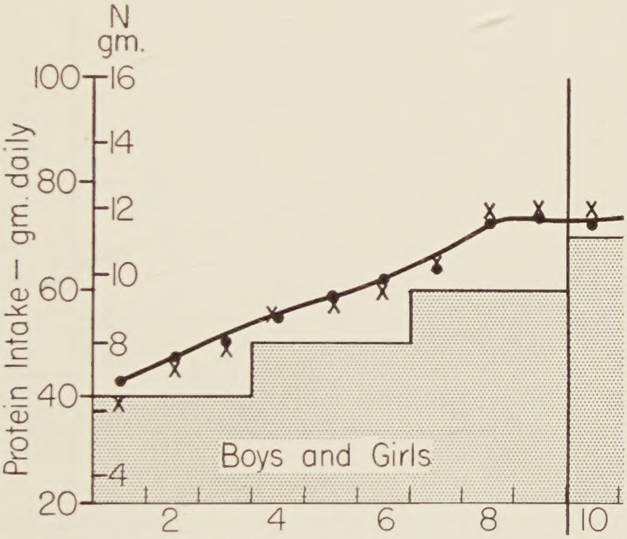

FIGURE 2. Mean protein intake of children of this study compared with the Recommended Nutritional Allowances.[2] The dots represent the protein intake as estimated from the United States Department of Agriculture Handbook No. 8;[3] the crosses represent protein intake calculated from nitrogen analyses of mixed diets.

The pattern of bodily growth changes conspicuously during the period of slowing growth. The rate of growth in length of the extremities is greater than that of the trunk. Such physical changes as these must be translated into terms of tissue growth to determine the child's requirement for each nutrient.

The largest single tissue of the body is the skeletal musculature. According to Scammon's anatomical studies,[4] skeletal muscle composes 25 per cent of the body weight at birth and throughout infancy. By or before 12 years of age, the quantity of skeletal musculature has increased to its adult proportion, that is, to about 45 per cent of body weight.[4] Thus, at some time during or perhaps throughout early and mid-childhood the musculature must grow at a rate far more rapid than that of the rest of the body for its total quantity to increase at nearly twice the rate of increase in total weight.

The rate of growth of other tissues is more difficult to measure. It is known, however, that considerable skeletal musculature can be sacrificed during periods of protein deprivation to prevent loss of protein from more important tissues. It can be assumed, therefore, that the protein requirements of other body tissues have been met if the skeletal musculature is well maintained. The rate of growth of the skeletal musculature will thus be the dominating factor in determining the protein requirement of children.

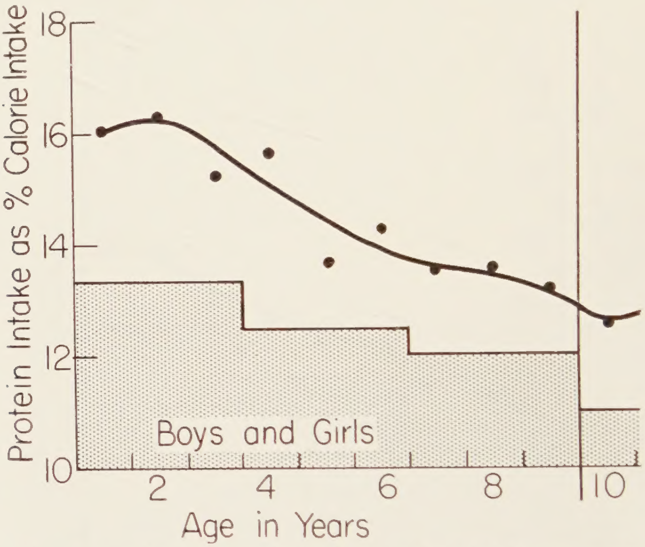

FIGURE 3. Protein intake, as percentage of total caloric intake, compared with Recommended Nutritional Allowances.[2]

Urinary Creatinine

Increasing knowledge of the influence of the liver and the kidney in the manufacture and excretion of creatinine has overshadowed the fact that, in the normal person, the amount of creatinine excreted in 24 hours still is an excellent measure of quantity of skeletal musculature.[5] It also has been shown that, despite the high creatine excretion of infants, their 24-hour urinary creatinine per kilogram of body weight remains constant throughout the first year of life.[6] Also, the quantity of musculature of the infant, measured by quantity of urinary creatinine per kilogram, is consistent with the relative proportion of muscle to body weight in infancy, as observed by Scammon from anatomical studies.

It seems highly probable that children over one year of age will show the same characteristics as infants; namely, that each age of childhood will have a characteristic range of quantity of musculature, varying with the body build of a given subject. This characteristic value of urinary creatinine per kilogram daily for each age is chosen as representing the mean quantity of skeletal musculature normal for the age. The protein intake permitting the quantity of muscle characteristic for any age is considered the protein requirement for that age.

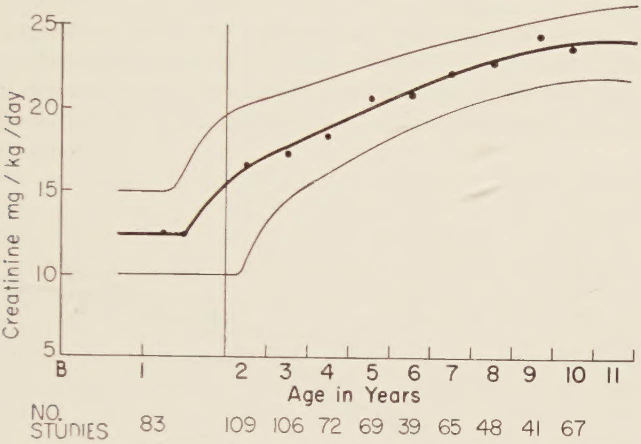

FIGURE 4. Mean creatinine per kilogram for boys of each age group studied. The fine lines represent standard deviation for the given age. This figure indicates relative growth of the skeletal musculature in relation to total body growth.

TABLE 1

MEAN DAILY URINARY CREATININE PER KILOGRAM OF BODY WEIGHT

Age	Sex	Number of studies	Mean creatinine per kilogram	Standard deviation
1	MF	83	13.5	3.1
2	MF	109	16.3	6.2
3	MF	105	17.4	2.5
4	M	72	18.3	2.0
5	M	69	20.9	2.2
6	M	39	20.9	2.0
7	M	65	22.3	2.1
8	M	48	22.9	2.0
9	M	41	24.7	1.9
10	M	67	24.0	2.1

In FIGURE 4 and TABLE 1 the mean 24-hour creatinine excretion per kilogram of body weight is shown for boys from 6 months to 11 years of age. The figure indicates the relative changes in proportion of skeletal muscle to total body weight, for the age range considered.

The excretion of creatinine during somewhat more than the first half of the second year is maintained at the same rate as that of the group of infants given undiluted cow's milk, some of whom also were included in this study. During the latter part of the second year the excretion rises with almost startling abruptness. The mean excretion has increased over the infancy value by about one third at the time of the third birthday. This remarkable increase in relative quantity of muscle occurs during the period of most marked slowing of growth in body weight and height. The growth of muscle accounts for about half the total weight gain for the year. The rate of increase slows somewhat after the third or fourth year, although the skeletal musculature continues to grow at a greater rate than the body as a whole until the child is about 9 years old when, diet permitting, the adult proportion of muscle to total body weight is reached. Girls at 10 years, the youngest age for which we have sufficient data, already have achieved approximately their adult proportion of musculature, which is somewhat lower than that observed for boys.

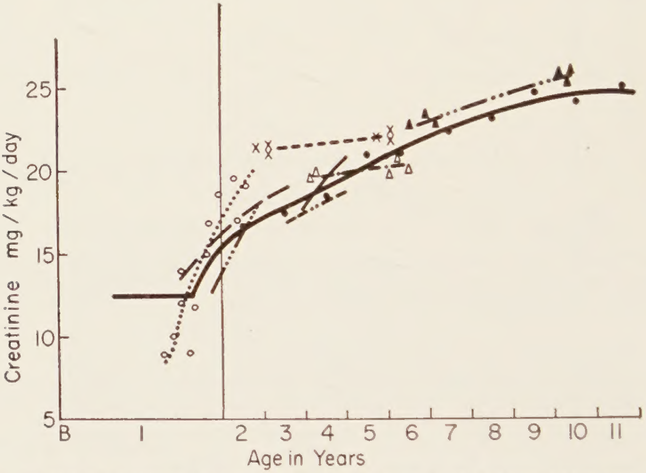

FIGURE 5. Rate of increase in creatinine per kilogram with increasing age as shown by different children.

If the protein intake does not permit muscular growth at the rate shown, the child does the best he can with the protein provided him. A protein intake above those used here does not increase the quantity of musculature significantly. The exact age at which increased rate of growth of muscle begins, and the rate at which it proceeds in the young child vary considerably from subject to subject. The creatinine excretion of many of our very young subjects was followed for periods of from 6 to 20 months; that of a few children was studied again after a lapse of 2 or 3 years. The rate of increase in per kilogram creatinine excretion is illustrated for the different children in FIGURE 5. For most of the subjects only the curve showing the rate of increase is shown. The actual maximum range of values for the separate studies is shown as circles for the youngest child, who came to us definitely undermuscled, though of normal weight, and increased his quantity of musculature very rapidly. The variation of values noted from study to study in this child illustrates the commonly observed variation in "constancy" among infants and very young children.

The slope of the lines of creatinine increase per kilogram of weight for each of the several children shows that, when protein intake is ample, both the age at which the skeletal musculature begins to grow more rapidly than the total body growth, and the rate of increase in muscle growth vary with the individual child. Each child studied exhibited this increase in rate of growth of muscle before 24 months of age. Some of the children appeared to have a period of very rapid muscular growth that slowed as sharply as it began; others showed a slower rise that continued for a longer period. It is certain, however, that the period from 18 months to 3 years and, perhaps, to 4 years of age is the period wherein growth of the skeletal musculature is most rapid in relation to total body growth.

It is perfectly natural that growth of musculature should proceed in this manner. The child learns to walk at about 1 year of age. By 18 months of age he spends many of his waking hours on his feet. Muscles of the back, buttocks, and legs must strengthen greatly. The stimulus to growth is provided, if food permits it. As his legs grow in length and his center of gravity is progressively farther from the floor, more muscle must be developed to maintain posture and permit activity. It is well that this period of increased rate of growth of muscle be completed before the period of rapid circumpuberal growth begins. The child will then need all of the protein he can retain for body growth, without the additional burden of incompleted musculature.

If the period of slow body growth is the period of most rapid growth of muscle tissue, it seems obvious that protein requirement will be high during this age range—probably as high as during infancy. It will be relatively high throughout the entire period wherein muscle growth is more rapid than total growth. The actual requirement at any age will vary with the rate of growth of muscle and with the child's efficiency of utilization of his intake.

Nitrogen was well absorbed at every age studied. The mean fecal nitrogen remained almost constant at about 11 per cent of the intake throughout the entire age range reported here. The nitrogen absorbed, but not retained, is excreted in the urine.

The various levels of protein intake were obtained largely through adjustment of the milk intake, substituting nonprotein calories for milk, as is commonly done in families.

The retention of nitrogen with increasing intake is shown on a per-kilogram basis for children 1 to 4 years of age in FIGURE 6 and in TABLE 2. For comparison, mean retention values are shown for infants given a similar range of intake. Mean retentions for 1-, 2-, and 3-year-olds are shown by separate symbols, averaged for each 50 mg. of increase in intake of nitrogen. No significant difference in retention for any given intake can be noted among the children of these 3 ages. The line of mean retention is drawn through the means for all data for each 25-mg. increase in intake. Standard deviation from the mean is shown in TABLE 2.

FIGURE 6 shows that the retention of nitrogen by young children is somewhat smaller and less steady than that of the infants given comparable intakes. It also shows that small children retain very little of a low nitrogen intake, but the retention rises rather sharply between 400 and 500 mg. nitrogen intake

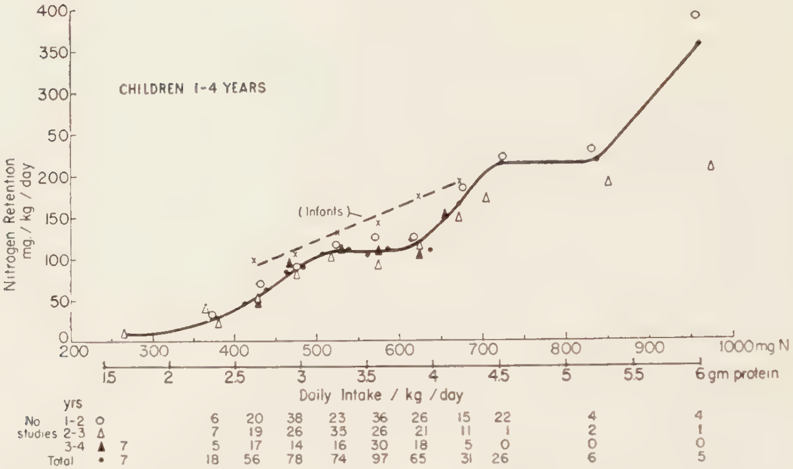

FIGURE 6. The retention of nitrogen per kilogram daily in relation to increasing per kg. intake. Data for infants are shown as broken lines; those for children from 1 to 4 years are shown as solid lines. The symbols differ for each year of age. The mean retention values were obtained by averaging the retention data for all 3 age groups for each 25 mg. of increase in intake.

TABLE 2
MEAN RETENTION OF NITROGEN IN RELATION TO INTAKE: CHILDREN ONE TO FOUR YEARS OF AGE*
(Data as Milligrams per Kilogram Daily)

Intake range	One-year-olds			Two-year-olds			Three-year-olds			Summary			
	No.	I	R	No.	I	R	No.	I	R	No.	I	R	SD
200–299							7	265	8	7	265	8	24
300–349	2	339	11	1	348	18	1	318	54	4	336	23	19
350–399	4	389	42	6	388	21	4	379	32	14	386	30	24
400–424	6	411	44	7	410	44	5	418	48	18	412	45	24
425–449	14	441	81	13	439	56	12	439	46	39	438	61	36
450–474	14	463	85	11	466	66	9	460	100	34	463	83	49
475–499	24	485	91	14	484	90	5	480	81	43	484	88	40
500–524	12	511	118	24	510	105	5	509	77	41	506	103	44
525–549	11	535	112	11	535	83	11	538	124	33	535	109	40
550–574	21	562	119	16	565	88	13	559	106	50	562	102	53
575–599	15	584	128	10	591	89	17	586	105	42	586	109	40
600–624	21	613	126	11	612	116	9	614	106	41	613	119	43
625–649	5	640	110	10	638	105	9	635	104	24	637	106	35
650–699	15	678	182	11	674	146	5	657	145	31	673	163	73
700–749	13	728	217	4	705	168				17	722	205	58
750–799	9	769	220							9	769	220	54
800–899	4	831	225	2	850	184				6	838	211	111
900–	4	955	381	1	974	210				5	959	347	191
Totals	194			152			112			458			

* Symbols: No. = number of studies; I = intake; R = retention; and SD = standard deviation of retention.

(2.5 to 3 gm. of protein) per kg., then apparently reaches a plateau, only to show a second rise when the intake is increased above 650 mg. (4.0 gm. of protein). It is emphasized that these data refer to intakes attained voluntarily by children given dietary regimens consisting of common foods normally eaten by these age groups. The intake of protein foods was not forced, nor was the diet "stuffed" by the addition of special proteins. Few of the children consumed amounts of protein over 4.5 gm./kg./day.

Nearly 80 per cent of the data lie between intakes of 450 and 650 mg. of nitrogen (2.75 to 4 gm. of protein) per kg. body weight. The mean retention for this entire group approximates 110 mg. kg. with a range of (approximately) 70 to 150 mg. kg. These retentions of nitrogen were adequate to permit growth of the skeletal musculature to the levels shown in FIGURE 4. An intake of 3 gm. of protein per kg. is considered the minimum permitting a satisfactory retention of nitrogen for children 1 to 4 years of age. An intake over 3.5 gm. kg. is no longer economical. An intake between 3.0 and 3.5 gm. kg., or approximately 1.5 gm. lb., seems a safe allowance for children 1 to 4 years of age.

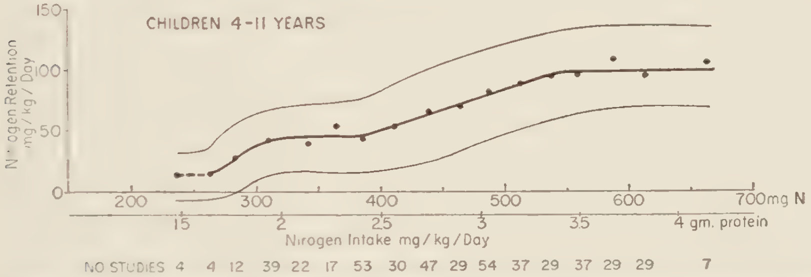

FIGURE 7. Retention of nitrogen per kilogram daily in relation to increasing per-kilogram intake; data for children 4 to 11 years of age. The mean retention is determined by averaging all retention data for each 25 mg. of increase in intake of nitrogen.

The curve of mean per kilogram nitrogen retention of 4-year-olds given increasing amounts of protein differed significantly from that of children under that age. Children 4 through 10 years of age showed no significant differences in mean quantity of retention for a given nitrogen intake. Therefore, the data for all children 4 through 10 years have been combined, and the mean retention per kg. daily is shown in relation to increasing per-kg. nitrogen intake in FIGURE 7. TABLE 3 separates the data into 2 groups; children 4 to 7 and 7 to 11 years, respectively. The last column shows the data for the combined groups.

Except for one group of 24 studies of 5-year-olds, whose mean nitrogen intake was 309 mg. kg., data for the 4- to 7-year-old children lie predominantly between 475 and 600 mg. of nitrogen per kg. of body weight. The nitrogen intake of children 7 to 11 years of age is distributed more evenly; about 80 per cent is between 325 and 525 mg. kg., with the total range of 225 to 661 mg./kg. With increasing protein intake, nitrogen retention of the 4- to 11-year-old children tended to increase stepwise, as did that of the younger group.

All showed some evidence of resisting loss of nitrogen at low intakes, though the data are insufficient to be conclusive in either group. The retention of the older group leveled off at 46 mg. of nitrogen per kg. of body weight between intake of 300 and 400 mg. of nitrogen per kg. of body weight (about 2 to 2.5 gm. of protein). With increasing intake of nitrogen, retention rose steadily, but at a slower rate than that observed in the young children. The second retention plateau at 100 mg. of nitrogen per kg. was achieved at intakes above 525 mg./kg. (3.5 gm. of protein per kg.), or about the same intake level as was necessary to provide 100-mg. retention for the young children.

TABLE 3
MEAN RETENTION OF NITROGEN IN RELATION TO INTAKE:
CHILDREN FOUR TO ELEVEN YEARS OF AGE*
(Data as Milligrams per Kilogram Daily)

Intake range	Four- to 7-year-olds			Seven- to 11-year-olds			Four- to 11-year-olds			
	No.	I	R	No.	I	R	No.	I	R	SD
200–249	2	245	31	2	227	−1	4	236	15	20
250–299	6	267	22	10	273	23	16	270	23	32
300–324	33	309	40	6	310	65	39	310	52	28
325–349	6	340	54	16	339	21	22	340	38	24
350–374	3	363	42	15	365	33	18	364	37	26
375–399	5	389	68	47	385	37	52	387	53	29
400–424	7	416	72	24	410	53	31	413	62	41
425–449	9	441	63	37	437	67	46	439	65	37
450–474	6	460	71	23	463	67	29	462	69	47
475–499	28	487	83	26	488	73	54	488	78	38
500–524	26	515	90	10	508	105	36	512	98	39
525–549	15	537	73	14	537	104	29	537	89	34
550–574	30	558	89	8	554	119	38	556	104	41
575–599	20	585	116	9	588	108	29	587	112	35
600–649	19	619	105	10	620	101	29	620	103	27
650–699	6	663	101	1	661	144	7	662	123	17
700–	1	715	130				1	715	130	
Totals	222			259			481			

* Symbols: No. = number of studies; I = intake; R = retention; and SD = standard deviation of retention.

When the retention curves for the 2 groups are compared, the chief differences observed are the increased retention of the older group as compared with the younger at intakes below 400 mg. of nitrogen per kg. of body weight, and their relatively slower rate of rise as intake increased over 400 mg./kg.; this made establishment of a precise requirement more difficult.

The relative rate of growth of musculature for children 4 to 10 years old still is greater than rate of growth of the body as a whole, although the difference in rates diminishes as the child approaches 9 years. On the other hand, annual rate of body growth is somewhat more rapid after 5 years, so that total growth of musculature is still rapid. In addition, these are the kindergarten and early school years, during which the common contagions are prevalent and the child develops his immunities. Bed rest alone lowers the retention of nitrogen, and loss may be heavy during a febrile illness. Such loss of protein tissue is regained far more slowly than it is lost. Return to prior weight may not be synonymous with return to previous quantity of protein tissue. Again, it appears wise to keep the requirement generous.

Each 100-mg. rise in intake from 300 to 600 mg./kg. includes about one third of the total data. The mean retention of those given 300 to 400 mg. was 46 mg./kg. One half of the children retained less, and one sixth retained less than 16 mg./kg. These less efficient children in this age range may suffer if the intake is limited to 2 to 2.5 gm./kg.

It is known that the orphanage diets provided protein in the amount of about 2 to 2.5 gm./kg./day. The children came to us with poor musculature. Those under 7 years maintained excellent musculature with dietary protein of 3 gm./kg. The children given 3 gm. of protein per kg. of body weight (slightly less than 500 mg. of nitrogen per kg.) retained an average of about 80 mg./kg./day. Two thirds of the group retained more than 45 mg./kg. A protein intake of 3 gm./kg. is easily achieved. Three glasses of milk, needed also for its calcium, one serving of meat of a size for the child's age, and 1 egg daily, together with the cereals and other vegetable proteins customarily eaten, will provide 3 gm./kg. for the younger children and 2.5 gm./kg. for the 9- and 10-year-olds. If the older children have the common after-school lunch of a glass of milk and a peanut-butter sandwich in addition to their 3 meals, their protein intake will reach 3 gm./kg./day.

An intake above 3.25 gm./kg./day is uneconomical for this age range. An intake of 3 gm. of protein per kg. for the 4- to 7-year-olds, decreasing gradually as the child grows older but keeping well above 2.5 gm./kg./day, will permit maintenance of growth of muscle and allow sufficient protein for recovery from such exigencies as febrile and other illnesses common to children. The children studied have shown no evidence that protein intake levels of 3 gm./kg. or more have any deleterious effect. We see no objection to maintenance of 3 gm. of protein per kg./day for the active school child. It is insurance against deleterious influences, and permits him to endure the stress of rapid circumpuberal growth without any added stress of nutritional deficiency. As protein foods are carriers of so many of the other nutrients essential for good health, generous use of protein is the cheapest nutritional insurance we can buy for children. Our evidence indicates overwhelmingly that such a diet is highly beneficial.

Summary

The skeletal musculature grows far more rapidly than the body as a whole from the age of 18 months to 9 years, or later if protein intake is insufficient to permit achievement of adult proportion of muscle to body weight by that age. Growth of muscle is most rapid during the preschool years, when weight gain is slow but type of body growth is changing and the extremities are growing more rapidly than the trunk. During those early years it appears advisable for the child to receive daily 3 to 3.5 gm. of protein per kg. of body weight; such an allowance permits good growth of musculature.

As the child grows older, if good nutrition has been maintained the differences

in rate of muscle growth compared to rate of total body growth become less. Protein allowances decreasing gradually from 3 gm. kg., but remaining above 2.5 gm. kg. will permit achievement of adult proportion of skeletal musculature to total body weight by or before 10 years of age.

The years from 4 to 10 are those wherein incidence of febrile illness is highest, so protein allowance must be large enough, not only for normal growth, but also to permit rapid recovery of muscle and other tissue inevitably lost during illness. A generous allowance permits better growth and maintenance of body tissue, and more adequate defenses against illness. Children so maintained exhibit abundant health.

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PROTEIN NEEDS OF CHILDREN: A PRELIMINARY REPORT OF STUDIES OF INDIVIDUAL DIFFERENCES*

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Children differ widely among themselves at any age in all aspects of health, growth, development, and adaptation. Many of these variables are known to affect protein as well as other nutritional needs, but little is known about the extent to which these factors do so, age by age, individually or collectively. Levine[1] has reviewed the principal factors on which a child's needs depend.

It is difficult to individualize the application of recommended allowances. The problem of recognizing those children whose protein needs are high or low because of basic attributes, or those who temporarily have such needs for special reasons, is difficult because frequent measurement of such needs in the laboratory is impracticable and because the early signs of unmet needs are nonspecific. Accurate clinical observation or measurement of those features of growth and development that are known to influence protein needs is likewise difficult.

This paper deals with a limited aspect of a project that has been in progress at the Department of Maternal and Child Health over the past 26 years under the title of "Longitudinal Studies of Child Health and Development."[2] In this project, individual children have been followed periodically† in a multidisciplinary manner from birth to 18 years of age, including limited studies of the mothers during the pregnancies and, thereafter, of the families and environments. Two hundred and thirty-two children were followed from birth to 6 or more years, 134 of these being followed to 18 years. The staff is now involved with the analysis of the extensive data in hand and in preparing both monodisciplinary and multidisciplinary reports of the findings. Primary emphasis is being placed on the recognition of individual difference throughout entire childhoods, in patterns of growth and development on the one hand and in dietary intakes, illness experiences, and environmental circumstances on the other, as well as upon identifying interrelationships between these data.

This paper constitutes a preliminary report on one small aspect of the research described, and it does so primarily from the standpoints of exploring

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* The research project from which the studies reported in this paper are derived has been supported in various aspects and in different years by grants from many sources. The major support for the areas of research dealt with here has been provided by: The General Education Fund of the Rockefeller Foundation, New York, N. Y. (1933 to 1943); The James Foundation of New York, New York, N. Y. (1944 to 1949); The Nutrition Foundation, Inc., New York, N. Y. (1945 to date); and the Research Grants Division of the Public Health Service, Bethesda, Md. (1947 to date).

† The routine followed provided interval histories and a series of examinations at birth, 2 weeks, 3, 6, 9, and 12 months of age, and every 6 months to 10 or 12 years, and yearly thereafter. The data regularly collected included body measurements, photographs, roentgenograms of several areas, pediatric, orthopedic, and dental evaluations, hemoglobin determinations, histories of illness experiences, dietary intakes, habits, activities, and psychosocial problems. Psychological tests and other special examinations also were included less regularly. Basal metabolic determinations were made only during the early years, and no biochemical or nitrogen-balance studies were included.

methodologies for evaluating longitudinal data and for studying interrelationships. It is concerned specifically with identifying relationships between size and growth progress and dietary intakes of calories and of total protein. These relationships are studied in individual cases, in respect both to continuing characteristics and to recognizable deviations at particular periods. The data selected for these purposes from the records in hand are:

(1) Direct measurements of body weight throughout, of length to 5 years, and of height thereafter.

(2) Measurements of breadth of subcutaneous tissue and of muscle at the widest breadth of the calf as obtained from anteroposterior roentgenograms. FIGURE 1 shows an anteroposterior roentgenogram of the leg with lines indicating the standard locations for obtaining the measurements of length of tibia, breadth of bones, breadth of muscle, including bone shadows, and breadth of the 2 widths of overlying skin and subcutaneous tissues.[3]

(3) Assessments of skeletal age according to the method of T. W. Todd, using roentgenograms of the hand and based on the norms published by Greulich and Pyle.[4] *

(4) Recorded age of maximum growth and of menarche in girls.

(5) Evaluations of usual daily intakes of calories and of total protein during each interval between examinations.

In subsequent reports it is planned to utilize additional data in search of other attributes that may be associated with characteristic differences in protein needs. These may include other body measurements and photographs to permit further consideration of the influence of body build, more detailed consideration of increments of growth, calculations of volumetric proportions of different tissue components, histories of illnesses, hemoglobin values, and selected items from pediatric, orthopedic, or dental examinations. Work is in progress along several of these lines.

Dietary Histories and Their Assessments[5]

Detailed histories of customary food intakes of each child enrolled in this study, covering each interval between examinations, were taken by one of the authors or by a specially trained nutritionist under her supervision. These histories provide quantitative estimates of average daily consumption of each category of food, amounts being reported in terms of common household measures. They were taken from the mother during the early years and, thereafter, directly from the school child or adolescent. Similar histories were taken at trimester intervals from the mother during the pregnancy concerned. Information was obtained, both on written forms brought to the clinic and by detailed questioning at interviews, at which a double approach was used to provide a check for inconsistencies.

These histories have been assessed for each child, covering each age interval as to amounts of calories, protein by principal sources, and individual minerals and vitamins. It is now possible, therefore, to recognize differences between children in over-all patterns of intakes throughout childhood, as well as inconsistencies or unusual shifts in intakes at special age periods in individual cases. Plans are being made for further analyses of these records of food protein intakes in respect to amounts of protein from various sources having qualitative differences.

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* These assessments were made by I. Pyle, co author of the atlas used for standards of reference,[4] to whom the authors are indebted.

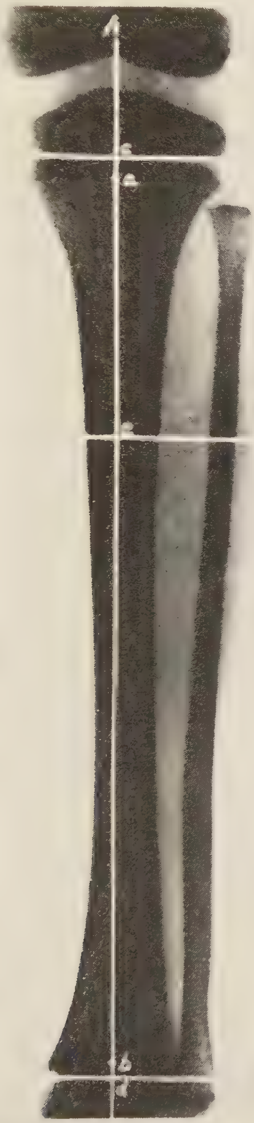


FIGURE 1. Roentgenogram of the leg area showing the method used for obtaining measurements of the breadth of subcutaneous tissue and of muscle in the calf of the leg.

The estimation of food intakes from dietary histories is subject to numerous errors, the most important of which is the accuracy of the reporting. The brief statement of the method adopted in this research indicates some of the precautions that have been taken to assure reasonable accuracy. Two of the authors have made tests of the reliability of the method adopted.[6] Satisfactory consistency in reporting has been found in comparing histories given in successive years by the same person. It is recognized that mother's histories may be consistently incorrect, but it has also been found that the report of intakes correlates with changes in growth progress.

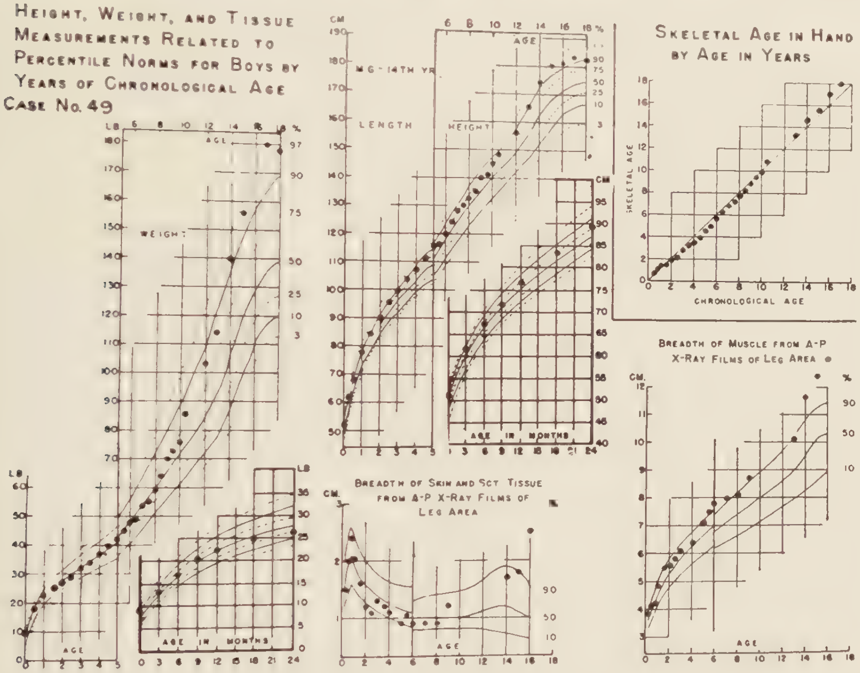
It is appreciated that dietary intakes, no matter how accurately they may be reported and calculated, do not establish actual needs. Inadequate intakes may be reflected in nonspecific clinical signs as well as slowed growth and lack of soft tissues, but these effects may not be recognized and, if recognized, they are still not specific. Excessive consumption of protein, associated with adequate caloric intake from other sources, may contribute to increasing obesity. It appears, however, that from the careful study of individual children over long periods, noting the ups and downs of growth and the levels of food intakes, indications may be obtained as to the probable extent to which intakes do or do not reflect actual needs, and limited inferences may be drawn.

Case Studies

Data derived from studies of 2 boys followed from birth to 18 years are presented in this preliminary report by means of charts. On the one hand, these data relate to physical growth and development and, on the other, to dietary intakes; they are plotted against selected standards of reference described in the presentation of the first case. An attempt is made to identify for each child his characteristic patterns of progress by noting the consistency with which he expresses his basic constitutional attributes and to distinguish deviations or irregularities suggestive of some factors interfering with expected progress. The fact cannot be overlooked, however, that consistently inappropriate progress may result from continuing nutritional fault or ill health. Comparisons of protein intakes by age with growth by age will be the principal focus of attention. The effects of illness experiences on these relationships are to be explored in a later report.

Case No. 49. FIGURE 2 shows a composite chart on which the measurements at all ages of weight, length to 6 years, height from 6 years, breadth of subcutaneous tissue, and breadth of muscle are plotted against percentile distributions and on which age of maximum growth is recorded. The norms for weight and length to 6 years were derived from the research being described; those for weight and height after 6 years are taken from norms provided by Meredith.[7] The norms for the 2 breadths were also based on the present studies.[3] Skeletal age in the hand is plotted against chronological age assessed in the manner described.[4]

The boy whose growth curves are shown in FIGURE 2 was above average in length and weight at birth (weight $8\,^{14}/_{16}$ lbs., length 51.6 cm.). He grew in height steadily, remaining between the fiftieth and seventy-fifth percentiles to 12 years. His rate of gain in height then accelerated so that he was at the ninetieth percentile by 13, and near the ninety-seventh at 15 and 16, and then tapered off to the ninetieth by 18 years. The weight chart for this boy shows considerably more shift in position over the years than did the chart for height. His weight fell somewhat behind during the first 2 years, then was more steady, but reached a low at the twenty-fifth percentile at 6½ years, and remained well behind height at all ages to 10 years. From that point it climbed steadily in the range, reaching the ninetieth percentile by 14 years, approximating the same position as height at that time. Between 15 and 16 years the boy became definitely overweight, but he thinned thereafter under voluntary dieting. This boy's adolescent acceleration, with early termination of growth in height and weight, coincided with a moderate advancement in skeletal development. His skeletal age fell behind to about 6 months of retardation at 5 years, then rose to near average at 7 years, remained near average to 13, and climbed thereafter to about 1 year of advancement at 16 years.


HEIGHT, WEIGHT, AND TISSUE MEASUREMENTS RELATED TO PERCENTILE NORMS FOR BOYS BY YEARS OF CHRONOLOGICAL AGE
CASE No. 49

SKELETAL AGE IN HAND BY AGE IN YEARS

BREADTH OF MUSCLE FROM A-P X-RAY FILMS OF LEG AREA

BREADTH OF SKIN AND SCT TISSUE FROM A-P X-RAY FILMS OF LEG AREA

FIGURE 2. Composite chart for Case No. 49.

The measurements of skin and subcutaneous tissue as plotted in FIGURE 2 reflect the major fluctuations in weight, being approximately at the ninetieth percentile at 1 year, near the tenth from 2 to 8, climbing to the ninetieth at 15 and well above it by 16. The measurements of muscle breadth, plotted similarly, indicate that, except in the first 2 years, this boy always had relatively large muscles in the leg, being at about the ninetieth percentile from 5 to 13 and well above it from 14 to 16 years. The latter increase in muscle breadth, in association with manifest obesity, may have been due in part to infiltration of muscle with fat.

Summarizing the findings in FIGURE 2, this boy was long and heavy at birth, moderately tall and relatively thin in late infancy and childhood, and then became relatively taller and progressively more heavy throughout adolescence up to 16 years. From photographs and clinical observations it was apparent that much of the change in his measurements reflected the development of a more stocky or mesomorphic build. However, he did have a large component of endomorphy in his build at 18 years and he became definitely obese for a short period centering around 16 years. None of this boy's illnesses appear to have been of sufficient severity or duration to suggest appreciable influence on his progress. In respect to his habits of physical activity, he appears to have been rather inactive during the early years and, for a boy of his build and excellent muscular development, he had surprisingly little interest in active sports during his second decade. It seems proper to conclude that during much of adolescence he was unusually inactive.

One might anticipate that an individual such as the boy described would have had relatively large protein needs at all ages because of his large size, but more so during adolescence because of more rapid growth, particularly of muscle tissue. One also might expect his needs for calories to be somewhat high. As to his actual intake of calories, one might suspect that they had been moderate and possibly low at times between 2 and 8 years, increasing progressively thereafter, and becoming excessive between 14 and 16 years.

The dietary records indicate that the mother's diet throughout pregnancy was good to excellent; her protein intake averaged 73 gm., but at times calories were excessive.

FIGURE 3 shows a preliminary distribution of calories and of protein from ages 1 through 6 years for the boys followed in this research. The most striking feature of these distributions is the wide range of variability at all ages. In each instance some boys consumed more at 2 years than others at 6 and, at every age, some took twice as much as others. It is also of interest that in each distribution the range increases with advancing age, with the exception of a range slightly wider between 1 and 2 years than between 2 and 3 years. This figure shows that the caloric intake of the boy under consideration rose rapidly during the period from 1 to 6 years, starting below the fiftieth percentile and rising above the ninety-seventh. The boy's protein intake started above the fiftieth and rose to the ninety-seventh percentile during the same period.

FIGURE 4 shows the calorie and protein intakes of this boy by years of age from 1 to 18, plotted against the recommended allowances of the National Research Council, Washington, D.C.. The line for the latter is drawn through the allowances plotted at the midpoints of the age periods to which they apply. It shows that his estimated intakes of both calories and protein were nearly double the allowances at 8 years and remained extremely high throughout. The peaks of intakes from 14 and 16 years coincided with his greatest growth and period of obesity. The findings, therefore, follow in general the expected pattern of change, but intakes at all ages were much higher than expected, especially during the preschool and early school years when weight gain was lagging.

This boy and his mother were both extremely cooperative in giving information and took their responsibility toward the study seriously. No scheduled examination between his birth and 18 years was missed. Therefore, it is hard to conclude that the histories of his eating practices were greatly and consistently exaggerated. It appears that his tolerance for calories and protein was relatively high for his age, for intakes were high even when he was rather thin in the early school years. It seems necessary to conclude that either he was able to consume over long periods far more calories and protein than he needed without getting fat or that he needed more of both than the recommended allowances of the National Research Council would have provided on an age basis. This would still apply even if full corrections were made on the basis of his height age or skeletal age. The case provides an example of individual variation in food patterns constantly high throughout childhood, associated with somewhat similarly unusual patterns of growth.

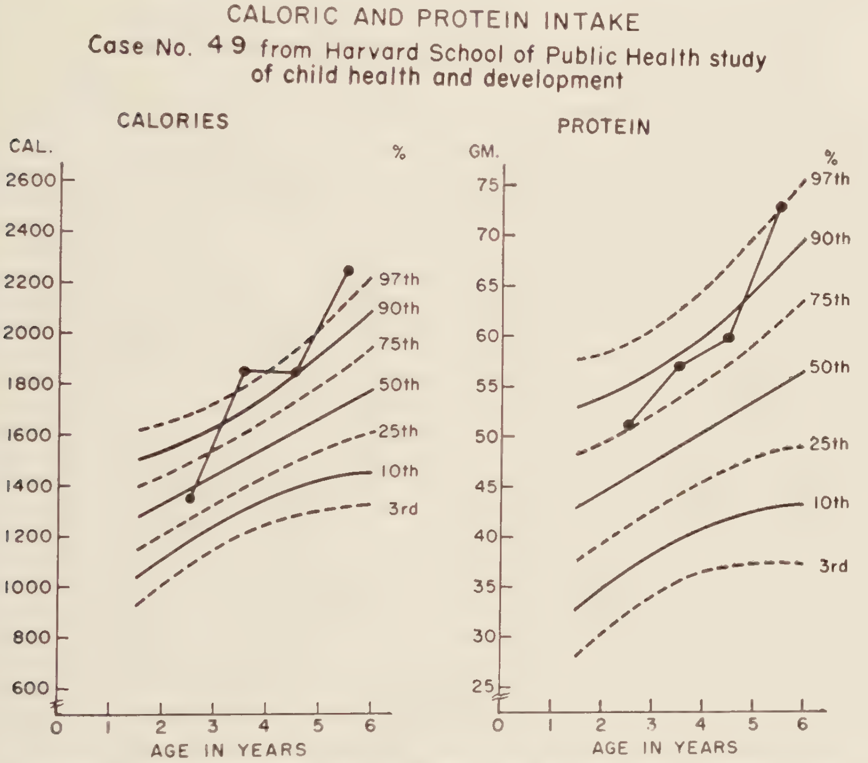


FIGURE 3. Calorie and protein intake for Case No. 49, plotted against percentile distributions for group aged from 1 to 6 years.

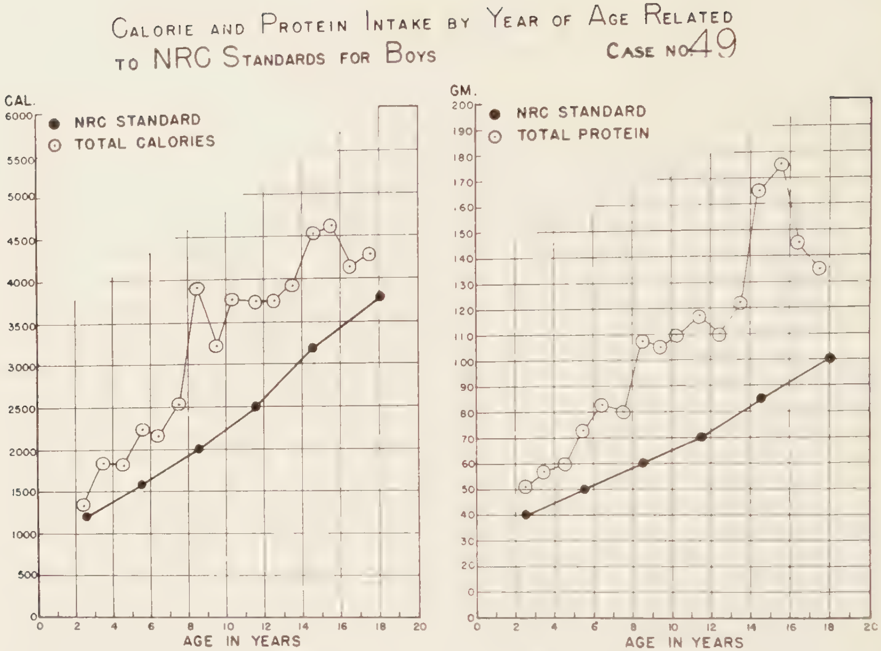


FIGURE 4. Calorie and protein intake for Case No. 49 plotted against National Research Council standards.

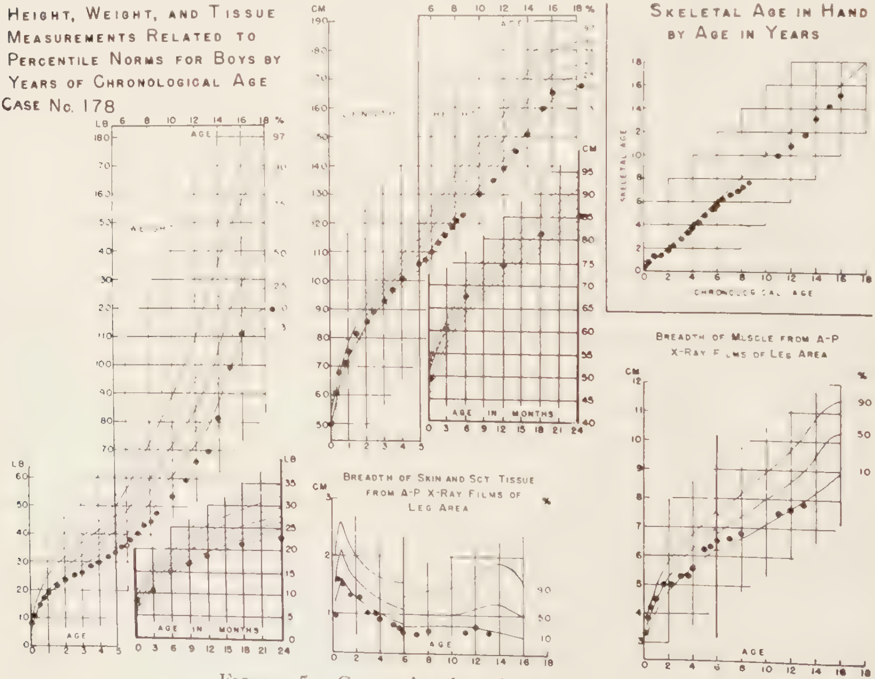


FIGURE 5. Composite chart for Case No. 178.

Case No. 178. The boy whose composite chart is shown in FIGURE 5 contrasts strikingly with the previous case in almost all the attributes portrayed, but the chief difference is in respect to size. He was very consistently at or below the third percentile for weight and subcutaneous tissues from 1 to 14 years, and between the third and tenth for height from 5½ to 14 years. His muscle breadth was near the tenth from 2 years on. The only substantial exceptions to his being small were that at birth he weighed 8 pounds and was 49.4 cm. in length, but the latter measurement was near the average through the first 6 months. Also, both height and weight climbed moderately in percentile position between 14 and 18 years.

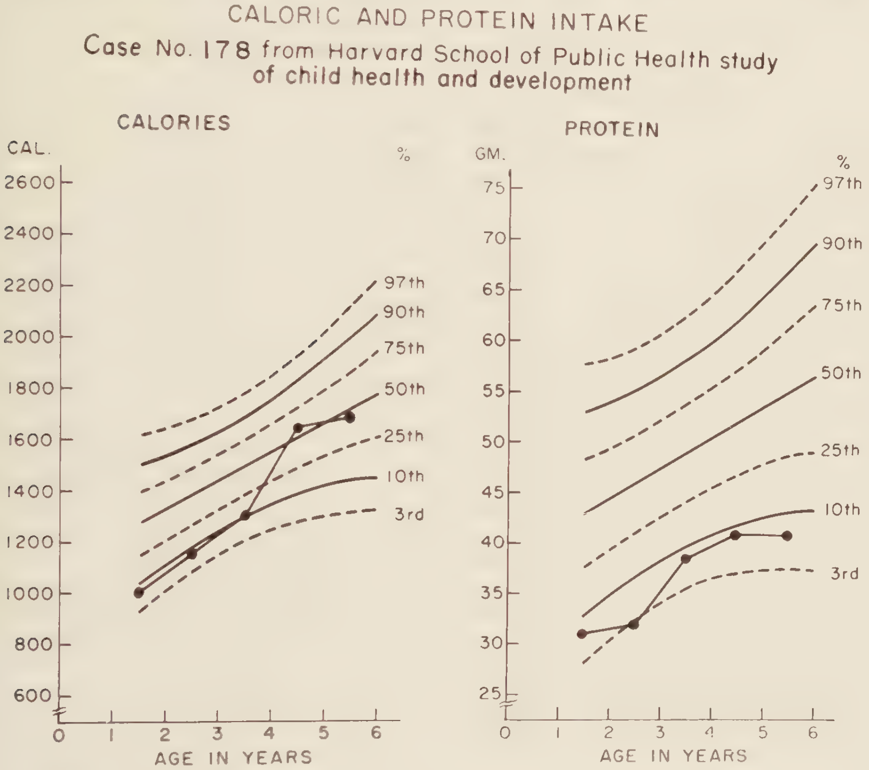


FIGURE 6. Calorie and protein intake for Case No. 178 plotted against percentile distributions for the group aged from 1 to 6 years.

The histories of the mother's prenatal diet were rated "fair" in protein. The baby was very slow in regaining birth weight, and the problem of feeding him was considered difficult throughout the first year. He continued to be finicky about eating and to refuse many foods until his ninth year; his appetite was usually considered "poor," or at best "fair." Tonsillar infections led to a recommendation for a tonsillectomy at 3 years that was not carried out until nearly 7 years. The whole period from 3 to 12 years was characterized by recurrent respiratory infections, some of which were severe in character.

FIGURE 6 shows that this boy's intakes of calories and protein from 1 to 6 years were very low in relation to the percentile distributions for the group under study. Although his calories did rise from the tenth to the fiftieth percentile between 1 and 5 years, protein intake never reached the tenth during these years. FIGURE 7, however, shows that his calories were never above the levels recommended by the National Research Council except from 3 to 5 years and, at times, they fell considerably below them. His protein intakes, however, were much more strikingly below these allowances at all ages to 14 years, being particularly low in respect to his own earlier and later intakes from 5 to 12 years. Thereafter they rose steadily to above the standard after 14 years.

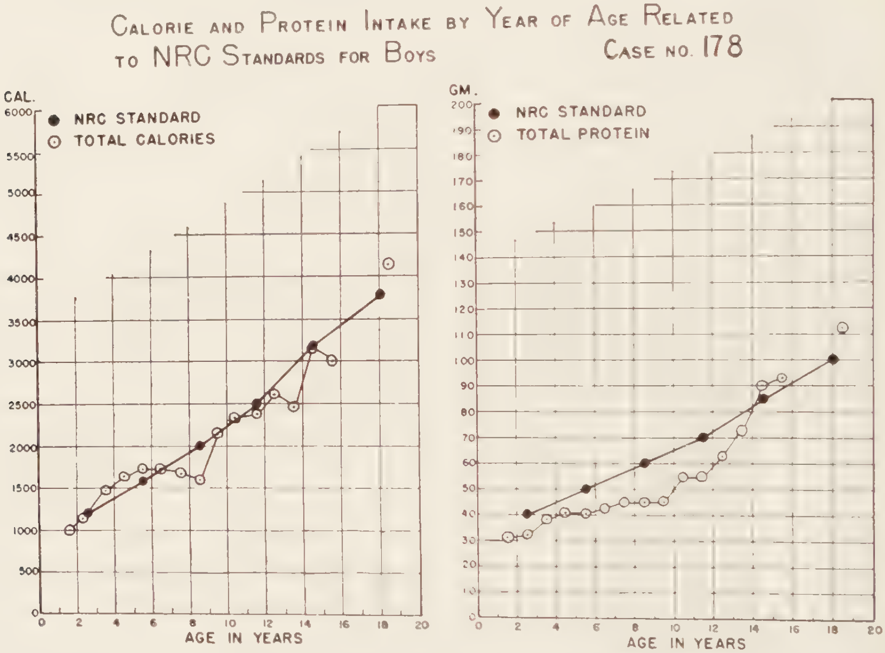


FIGURE 7. Calorie and protein intake for Case No. 178 plotted against National Research Council standards.

Between the ages of 9 and 10 years this boy, according to his mother, began to manifest an improved appetite, and at 14 years he was reported as enjoying his meals and eating much more than formerly.

This case appears to represent a consistently small child with weak potential for growth. These features would lead to the expectancy of low calorie and protein needs, and his intakes were generally low. During the years of his particularly low protein intakes his skeletal development was delayed and measurements of weight and soft tissues likewise fell behind.

Summary

The problems involved in estimating the long-term protein intakes of individual children and the possible relationships of these to the children's patterns of growth and development and to their particular needs have been considered.

Opportunities now available to the authors to view total childhoods in respect to growth and nutrition have been described briefly. Studies of two boys have been presented, using data obtained periodically over 18 years in each case. These data were acquired chiefly by a pediatrician and a nutritionist and were evaluated by a statistician. The data shown relate to each child's growth and development, on the one hand, and to his intakes of calories and protein on the other, as well as to possible relationships between these variables. Certain methodologies have been described for use in evaluating longitudinal data that have been found to assist in the recognition of differences between children and similarities or differences from age to age for the same child in levels of intakes of protein and calories and of patterns of growth and development.

In initial case studies it has been found that selected children tend to have characteristically high, moderate, or low intakes of both protein and calories, sometimes being very consistent in this over long segments of childhood. One of the cases discussed was able to consume levels of calories and of protein far above recommended allowances, while remaining relatively inactive and maintaining low weight for height, excellent health, and a steadily high level of progress in growth. This boy became obese only for a short period late in adolescence, during which his formerly high levels of intakes were greatly increased.

In contrast, the second boy was always small and he continually consumed small intakes of both calories and protein. Both of these intakes were very low in comparison with the group from this study and protein was particularly low in respect to National Research Council allowances. The latter applies chiefly to the ages 6 to 12 years. Progress in skeletal development became slowed during this period. There were numerous clinical indications that his health was only fair during these years, and he manifested generally lowered resistance to infections.

There do appear to be recognizable relationships in the cases presented between over-all patterns of growth and characteristics of calorie and protein intakes. No inferences can be drawn as to the significance of demonstrable deviations in progress associated with changes in levels of protein intake until the entire series has been studied. However, intriguing fluctuations occurring concurrently in growth and food intake suggest that further studies along these lines are desirable.

Individual differences in the characteristic levels of calorie and protein intakes appear to be wider than had been anticipated, to the extent that recommended allowances often bear only remote relationships to the customary intakes of selected individuals.

This paper is not concerned with basic protein needs of populations of children by age or otherwise. The authors have used dietary intake histories as a part of the broad study of human variability in childhood. They have pointed out only the wide discrepancies between individual intakes and recommended allowances. They have not been interested in the critique of recommended allowances for average children.

These findings suggest that it may be more appropriate to consider the adequacy of an older child's intakes in terms of his previous customary levels of intakes while enjoying good health, or in terms of his progress in growth, than in relation to standard allowances.

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PROTEIN REQUIREMENTS OF ADOLESCENTS

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The problem of the "optimal" intake of protein in the human subject involves considerations other than the satisfaction of the growth requirement. Longevity, maximum total efficiency, reproduction, and development of offspring were included as criteria by Slonaker[1] in his study of the rat, but these would be difficult of attainment in any longitudinal human study. However, his findings are of interest. With calories constant, 5 different levels of protein intake were used: 10.3, 14.2, 18.2, 22.2, and 26.3 percentages of total calories. The criteria of optimal growth, highest fertility, lowest mortality of offspring, and length of life were all best satisfied on the second level of intake, 14.2 per cent of calories. Spontaneous activity was optimal between 14.2 per cent and 18.2 per cent. These objectives are difficult to attain in any longitudinal study of the human. In the past 30 years studies of the relation of the protein intake to resistance to infection have yielded sufficient information to constitute another criterion of "optimal" in any consideration of protein intake. A series of studies by Cannon[2] led to the conclusion that "both the acquisition and preservation of the antibody mechanism require the dietary presence of an adequate supply of proteins of good biological quality in order to make available the building stones essential for globulin synthesis." Our own studies along this line have involved 2 disease states: tuberculosis[3] and rheumatic fever.[4]

In following, for more than 20 years, a group of 932 tuberculin reactors removed from contact, a significant number of instances of the adult form of the disease was noted first in the adolescent group. It was possible to demonstrate good correlation between the development of these lesions, their course when once developed, and the nitrogen metabolism. Evidence of previous depletion, not appreciated when estimates of nutrition were based on weight, but inferred from abnormally high nitrogen retentions on adequate intakes with flat or declining weight curves, was noted repeatedly. The typical metabolic picture of recovery from depletion was a high nitrogen storage with a plateau in weight curve, following which nitrogen storage fell to the expected plane and was paralleled by normal weight gain. Negative nitrogen balances from any cause were associated with spread of the disease process; regression of the pulmonary lesion was associated with normal or high retentions. Examples of these studies are reproduced in FIGURES 1 and 2.

In a series of studies on children with rheumatic fever, good correlation could be established between the nutritional state, with particular reference to protein reserves, and the ultimate outcome of the disease. Similar findings are reported by Coburn and Moore.[5]

The approaches to a study of the protein requirement in the human have been the survey of intake method and the balance technique. The former has the obvious defect of measuring intake rather than utilization, but it has broad usefulness when correlated with growth over long periods. It has the further defect of giving us no information on the composition of growth, something of prime importance in relating nutrition to resistance to infection.

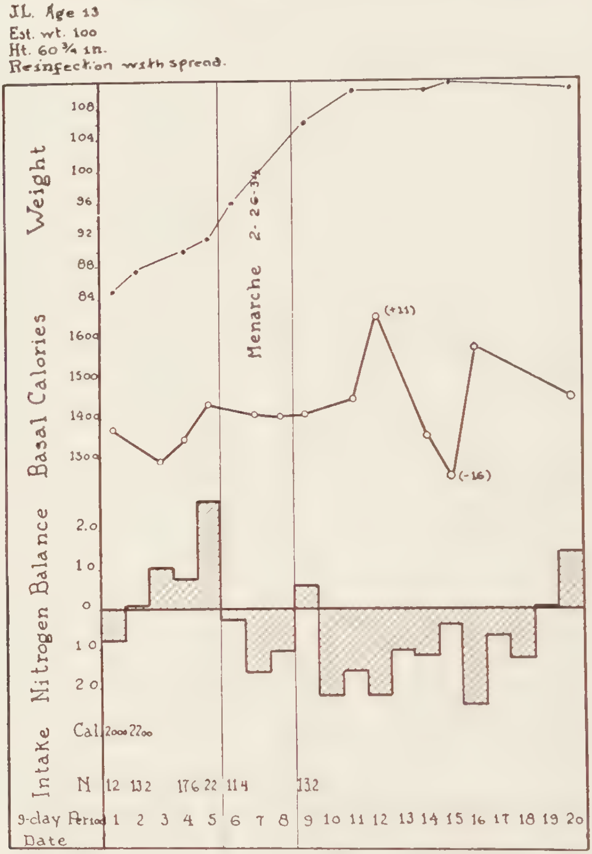

FIGURE 1. A 13-year-old girl was admitted to hospital with a minimal reinfection-type tuberculous lesion and was studied to determine the nutritional requirements at this age. In the first 45 days there was a steady gain in weight, but a positive nitrogen balance was maintained only with high intakes of nitrogen. The negative balance at the menarche could be attributed to a diminished intake, but immediately following the menarche the balances were negative on amounts (13.2 gm.) that gave positive balances before menarche. At this time a frank spread of the tuberculous lesion, requiring collapse therapy, was detected. A fall in nitrogen retention is noted regularly in girls at this point, so that marginal intakes resulting in a positive balance in the prepuberty period may result in negative balances at the menarche.

The phenomenon of a failure to gain in weight for a period of as long as 108 days while nitrogen was being accumulated in large amounts would not be noted with such an approach; conversely, gains in weight with negative nitrogen balances that we have noted to be associated with spread of tuberculosis would be obscured. On the other hand, short balance periods would give misleading information in that they would reflect previous deficits by large retentions. Very long balances probably do carry with them a systematic error, possibly in failing to include sweat losses, so that the gains in weight conventionally

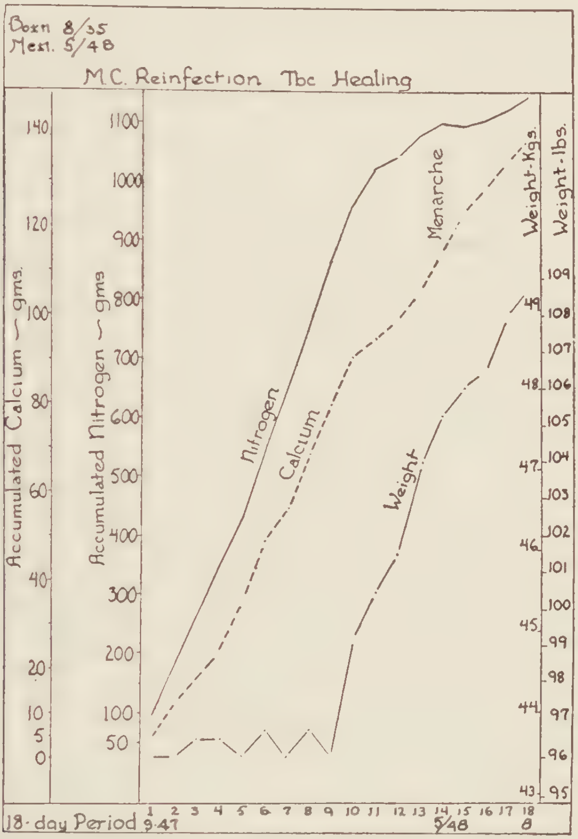

FIGURE 2. A 13-year-old girl, with a history of low protein intake, under treatment for a minimal reinfection-type tuberculosis. The disease spread while she was on her own self-selected diet, which was low in protein. The balance study was done while the girl was on a diet that derived 15 per cent of its calories from protein. For 162 days, during which she retained 865.7 gm. of nitrogen, the weight curve was flat, reflecting the situation seen when depleted stores are being replenished. During the next 162 days she stored 275.7 gm. of nitrogen while gaining 5800 gm. of weight. During the 18-day period following the first menstrual period, the nitrogen balance actually became negative.

associated with certain nitrogen increments do not correlate well. Losses incidental to menstruation have been checked and would seem negligible with periods of average duration; that is, approximately 0.8 gm. in a 5-day period requiring 9 pads. However, this conventional estimate has no basis, insofar as we know, for the composition of tissue of the rapidly changing adolescent. A further error will result if dietary intake is calculated from food tables rather than from daily analysis. In checking complete diets for 50 days, it was found that the nitrogen content exceeded the intake calculated from the Atwater and Bryant tables[6] by 11.75 per cent. Dietary surveys based on such calculations rather than on direct analysis would be subject to this error. Whether the actual retention noted on intakes of 15 per cent of the calories as protein are unrealistically high, the fact remains that with lower intakes we found actually negative balances too often to have any sense of security with lesser intakes. The discrepancy between weight actually gained and that predicted from nitrogen storage in the tuberculous adolescent is readily understood as a phenomenon of the reconstitution of depleted nitrogen stores. Even in the normal adolescent, however, the exchange of fat and water for protein in the rapidly changing structure of this age period may account for part of it. We lack data on the composition of tissue at this age period that would make valid the conventional prediction.

As a background to a consideration of the special problem of the adolescent, I propose to review some basic principles of the general problem of the protein requirement derived from balance studies on 27 children[7] on whom the length of observation averaged 10 months. These included 7 boys aged 10 to 13 years and 6 months and 5 girls aged 10 to 12 years and 5 months.

Although hospitalized for the study, these were normal children who would otherwise have been in foster homes. They attended school daily and had outdoor exercise. The criteria used for the adequacy of their intakes were (1) expected gain in height and weight, (2) satisfaction of appetite, (3) nitrogen retention, and (4) a normal basal metabolism. The caloric intake was varied until appetite was satisfied, and then the percentage of calories derived from protein was varied from 10 to 25 per cent. Consideration of protein requirement is inseparable from total caloric intake; this explains our recording of protein as percentage calories rather than as grams per unit of weight. This point is well made by Cathcart[8] and by Powers.[9]

Adjustment to a suboptimal intake of calories and protein is regularly made by a fall in basal metabolism.[10] This, together with its implications, was long since pointed out by Benedict.[11] Thus, while it is known that a subject on lower intakes of protein may come into nitrogen equilibrium at a lower plane, some thought must be given to the price paid for doing this at lower levels of basal metabolism (FIGURE 3). In the girl, cessation of menses is one of the side effects. Inability to cope with infection is an additional consequence. This was our reason for including as one criterion of "optimal" a normal basal metabolism.

Attempts to effect an increase in metabolism above normal with excessive amounts of protein were unsuccessful. In these studies there was a suggestion of an instinctive regulation of intake in that amounts of protein in excess of 20 per cent of the caloric intake were commonly refused. When taken, they frequently resulted in a syndrome of pallor and nausea. A third finding was the demonstration of the phenomenon of the "physiological ceiling" for nitrogen, excessive amounts of nitrogen being re-excreted in the urine.

The generalization from this study was that the criteria in question were regularly satisfied only with a protein intake providing 15 per cent of the otherwise adequate caloric intake, the remaining calories deriving 35 per cent from fat and 50 per cent from carbohydrate.

Consideration of the adolescent's requirement requires a brief review of the metabolic and growth characteristics peculiar to this period. The adolescent period will be considered here as embracing the span beginning with the appearance of secondary sex characters and terminating with the cessation of skeletal growth. Common usage makes "puberty" synonymous with the appearance of the first menstrual period in the girl; a point developmentally corresponding to this in the boy is the time of appearance of the spermatozoa, which is believed to coincide with the development of curly pubic hair. Chron-

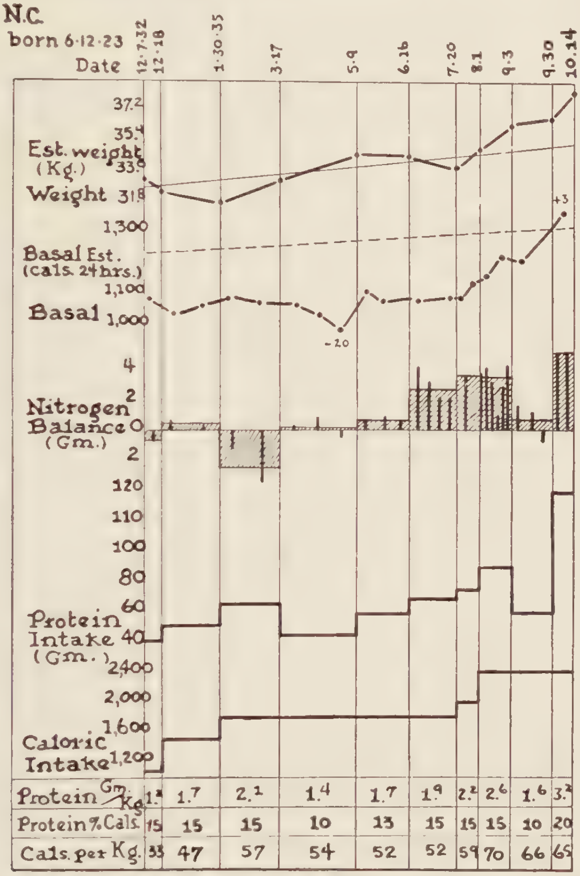

FIGURE 3. The effects of suboptimal intakes of nitrogen. Weight may continue to rise with a protein intake inadequate for normal retention. Part of the adjustment involves a depression of the basal metabolism. An increase in caloric and protein intake was followed by a rise in the basal metabolism rate from −20 to +3 Boothby. In this study the balances were not continuous; each bar represents a 3 day balance, and the cross hatched blocks show the average of the contained bars.

ologic age as a point of reference in this age group has little usefulness. The achievement of puberty has a range of about 6 years in both sexes, and the metabolic changes that characterize the period correlate with physiologic rather than chronologic age. The pattern of growth in girls is well brought out by Shuttleworth[21] in the plotting of height increments of girls menstruating first at 8 different chronologic ages (FIGURE 4).

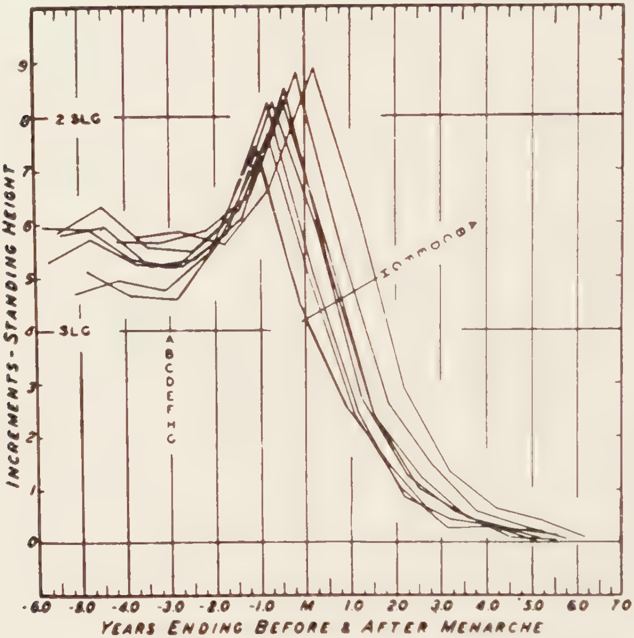

FIGURE 4. Growth in height during adolescence. This chart from Shuttleworth[21] brings out the fact that the growth patterns of girls maturing at ages from 10 to 17 are similar only when plotted in terms of physiological age. Shown are the annual increments in standing height of 8 groups of girls who attained menarche at different ages. The curves are arranged so that the points corresponding to the menarcheal age are on the same vertical line. The 3 horizontal grid lines indicate, respectively, zero growth per year, one ninth of the average growth from age 8 years to age 17 years (SLG), and two ninths of the average growth from age 8 years to age 17 years (2SLG). The decelerative phase of growth following the menarche is reflected in a diminishing ability to retain nitrogen. (Reprinted with the permission of Charles C Thomas & Co., Springfield, Ill.)

A prepuberal rise and a postpuberal fall in basal metabolism have been noted by some investigators, and my associates and I have noted it regularly in determinations done every 6 days. In cross-section studies, and with determinations at long intervals, the averaging process obscures this, as will be noted in the observations of Shock.[22]

With intake constant, the percentage of nitrogen retained rises during the accelerative phase of growth that precedes puberty and falls during the decelerative phase (FIGURE 5).

The magnitude of the fall in retention noted at puberty would seem disproportionate to the changing rate of growth, and it suggests a more positive hormonal influence. In 6 girls, the administration of estrogens exerted such a depressing influence on the retention of both nitrogen and calcium.[23] It is

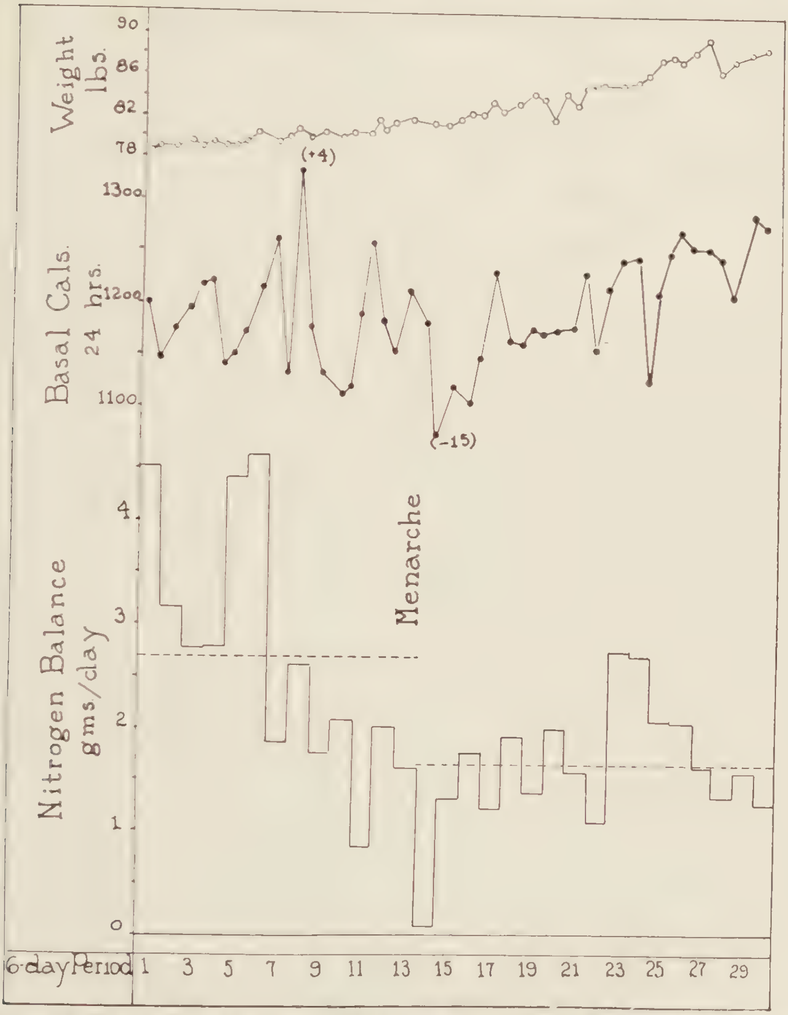

FIGURE 5. Metabolism at puberty. The basal metabolic rate is fluctuant in this well-trained subject, the range of basal calories being 200. There is a tendency to high rates immediately before menarche and to lower rates thereafter. The rates are calculated on Boothby standards. The period surrounding the menarche is marked by a fall in nitrogen retention, although intake remains constant. With amounts lower than 15 per cent this tendency actually results in negative balances.

probable that positive balances will occur with amounts lower than this during the accelerative phase of growth when the tendency to retain nitrogen is enhanced; it is during the period of decelerative growth that we feel that amounts lower than this will result in negative balances. That this may be a major factor conditioning the development of the reinfection type of tuberculosis is, we feel, implicit in the data. In 5 boys to whom testosterone was given, the well-established increase in nitrogen was noted, but there was a reciprocal fall in calcium.[24]

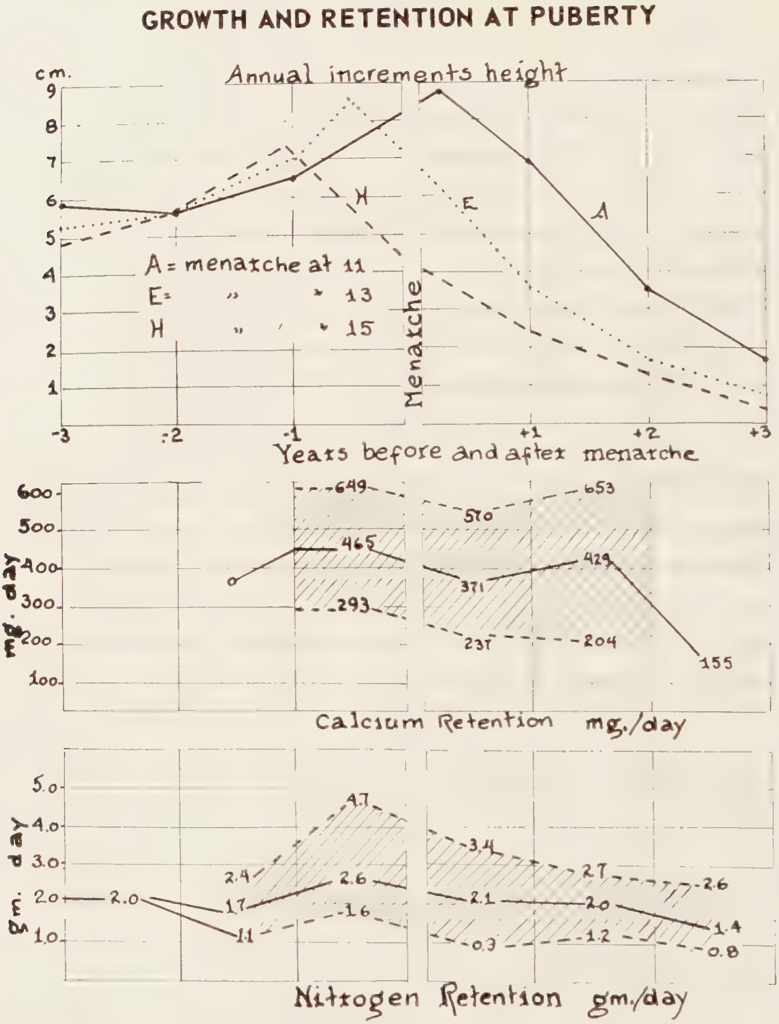

FIGURE 6. Data on 8 normal girls. The means are close to those of a larger group studied over shorter periods by Wang, Hodgen, and Wing.[15] Caloric intake, ranging from 1900 to 2400 calories, was 15 per cent phosphorus, 35 per cent fluorine, and 50 per cent carbon.

FIGURE 6 and TABLE 1 show data on 8 normal girls in the premenarcheal and postmenarcheal periods whose intakes of protein constituted 15 per cent of the total calories.[1] Mean retention rises in the so-called MG year* to 2.6 gm. of nitrogen and 465 mg. of calcium and declines by the third postmenarcheal year to a mean of 1.4 gm. of nitrogen and 155 mg. of calcium per day.[21]

Six 18-day balances on 3 boys and 3 girls with epiphyseolysis are recorded in TABLE 2. These balances are included here as examples of a condition limited to the year of maximum growth and occurring characteristically in the overweight child or in one whose growth in height has been abnormally rapid. It was shown that the abnormally low calcium balances could be rendered positive with the addition of vitamin D.[25] In 4 of the 6 children the retentions of nitrogen were lower than in our normal children on intakes of protein equal to 15 per cent of the calories, suggesting that even this figure may be marginal in instances of very rapid growth.

Table 1

Case	Age	Years before or after menarche	Weight kg	Height cm	Basal calories	Diet calories	Nitrogen intake	Percentage of calories as protein	Nitrogen retention	N × 6.25
1	13–4	+4½	52.6	157	1241	2200	14.8	16.8	3.41	21.30
2	13–2	−3/12	52.6	157	1395	2200	14.2	16.1	3.13	19.50
3	13–9	+1–4½	53.9	151	1349	2400	14.9	15.5	1.40	8.75
4	10–7		50.0	157	1296	2200	12.9	14.6	1.45	9.07
5	9–3		31.9	140	1122	1900	12.0	15.8	1.16	7.25
6	11–1	−1/12	44.5	160	1365	2200	14.1	16.0	4.71	29.50
	11–7	+2/12	43.5	160	1306	2200	14.2	16.1	2.57	16.10
7	11–6	−6/12	52.7	158	1304	1900	12.5	16.5	2.02	12.60
	12	0	50.0	158	1223	1900	12.5	16.5	1.12	7.00
8	9–1	−2/12	36.4	135	1187	1900	12.4	16.4	2.69	16.80
	9–3	+1/12	38.9	137	1201	1900	12.4	16.4	1.57	9.82

Table 2

Epiphyseolysis

Age	Height (inches)	Weight (pounds)	Food calories	Nitrogen		Calcium		Basal calories (24 hr.)
				Intake	Balance	Intake	Balance	
Boys								
14	64	133	2400	15.10	1.65	1.311	−0.014	1580
13	58	78	2200	12.16	2.68	1.161	+0.113	1119
14	68	146	2400	14.84	3.72	1.399	+0.086	1688
Girls								
13	62	104	2200	13.82	0.04	1.210	+0.175	1350
11½	58	87	1900	12.74	0.76	1.266	−0.039	1317
11	60	93	2200	14.58	1.88	1.232	+0.063	1390

Excellent reviews of the literature on the adolescent requirement are available in the papers of Wait and Roberts[17] and of Wang, Hodgen, and Wing.[18] Their findings and those of others are summarized in TABLE 3. It will be noted that the percentage calories from protein found in recent dietary surveys, as well as those recommended by National Research Council, are lower than the percentage we suggest, although our figure is in agreement with that recommended by Wang, Hodgen, and Wing,[18] by Holt and Fales,[16] and by Camerer[19] and Tigerstedt.[20] The retentions noted in the balance studies of

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* Year of maximum growth.

TABLE 3

Reference	10 to 12 years		13 to 15 years		16 to 18 years	
	Diet calories	Percentage protein calories	Diet calories	Percentage protein calories	Diet calories	Percentage protein calories
National Research Council (1953)						
Boys	2500	11.2	3200	10.6	3800	10.1
Girls	2300	12.1	2500	12.8	2400	12.5
Canada (1954)						
Boys	2333	10.1	2940	12.2	3480	6.9
Girls	2510	10.0	2500	11.8	2385	8.8
Young and Pilcher (N.Y., 1950)[12]						
Boys	2557	13.3	3654	12.0	3375	11.6
Girls	2275	12.3	1945	12.7	1976	13.0
Warnick, Brung, and Woods (Idaho, 1955)[13]						
Boys			2088	14.0		
Girls			3145	13.5		
Eppright, Sidwell, and Swanson (Iowa, 1954)[14]						
Boys			2553	11.3	3419	11.9
Girls	2381	12.5	2514	11.9	2552	11.7
Odland, Page, and Guild (Montana, 1955)[15]						
Boys			3150	13.9		
Girls			3050	8.7		
Holt and Fales (1921)[16]						
Boys	2525 14 children	14.5	3432 3 children	14.4	3922 3 children	12.8
Girls	2523 8 children	12.8	3258 4 children	8.2	3057 2 children	14.3
Wait and Roberts (1933)[17]						
Boys						
Girls		12.4		12.8		
Wang, Hodgen, and Wing (1936)[18]						
Girls			2279	16.6		
Camerer[19]	proposed 17 percentage calories as protein from age 5 years through growth period.				not included in averages below	
Tigerstedt[20]	showed 16 percentage calories as protein during adolescence in girls.					
Averages:						
Boys	2479	12.3	3003	12.6	3599	10.6
Girls	2398	12.0	2649	13.4	2474	12.6

Wang, Hodgen, and Wing are essentially the same as those noted in our normal children.

Conclusions

Our data suggest that the optimal intake of protein for the adolescent should constitute 15 per cent of an adequate caloric intake. It is suggested that the recommendations of the National Research Council for this age group be revised upward. It is urged that one criterion of "optimal" be the relation of a nutrient to infection.

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Discussion

Paul György (*University of Pennsylvania Medical School, Philadelphia, Pa.*): From a worldwide viewpoint, the question of protein requirement is of paramount importance, as shown later in this monograph in connection with the first public health problem in the world: kwashiorkor, or protein malnutrition.

Since the age classes beyond the first year of life already have been discussed, I propose to fill a gap by returning to a consideration of the newborn and the first twelve months of life.

The newborn and the young infant present the simplest problem in protein requirement. From the teleological point of view, which I still consider better in this connection than any scientific approach, it is my confirmed opinion that human milk is *the* food for the human infant and that, accordingly, the amount required to cover the caloric requirements, as determined by the classic workers in the field, Rubner and Heubner (Czerny and Keller[1]), must also cover the protein requirement. The protein requirement in the infant, therefore, should be and will be about 1.6 gm. kg. I refer, of course, to the protein in human milk. I do not think we can state the protein requirement categorically without indicating just what kind of food we are discussing: not only the protein, the nature of the protein, and the biological value of the protein, but the protein in its context with the food as such. When we speak of human milk we know the relation of calories, which is about 6 to 8 per cent. We know the nature of the fat and the nature of the carbohydrate, both of which probably are very important in determining the protein requirements.

During the last two years two international conferences have dealt with the problem of protein requirement: one in Princeton, N. J.,[2] and a second in Rome, Italy.[3] The volume resulting from the latter conference leads to practical conclusions. The authors of that report have selected, rightly or wrongly, a so-called reference protein based on paper work and have computed the proper amino acid pattern, which perhaps should apply to all age classes. I must admit, in this connection, that I do not like to improve on nature. Furthermore, I do not like a reference pattern that cannot be used in practice as standard. In the past, reference standards have been used widely in the vitamin field. Be that as it may, the proposed ideal reference protein is almost identical with the protein in human milk.

In the previously mentioned report of the Food and Agriculture Organization of the United Nations (FAO) on protein requirements, a curve appears giving the figures for protein requirements over all age periods. Based on the hypothetical ideal reference protein, the protein requirement of infants is stated to be about 2.25 gm. During the first two years these figures drop to much lower levels (1 to 1.5 gm.) and, with a slight elevation during adolescence, they reach the level of 0.4 gm. for adults. Compared with the figures presented earlier in this monograph, the amounts recommended by the FAO are much lower, which is fortunate, as we are considering a worldwide problem. If the FAO figures are correct, then the protein requirement for the world need not be increased, as would be the case if we take the figures of Stearns, for example.

The ratio of calories to protein is one of the most important problems, as emphasized elsewhere in this monograph by investigators of the kwashiorkor problem, and we cannot speak about protein needs without taking calories into consideration. It is better not to speak of the amount of protein per kilogram daily, but to express it as the percentage of the caloric intake.

Furthermore, as I have already mentioned, the carbohydrates are definitely important factors that influence protein needs or protein utilization. In milk, for instance, lactose, in studies that are now completed in our clinic,[4] has proved far better in protein utilization by infants than have other carbohydrates, such as dextrimaltose. Similar observations are derived from studies on animals. I repeat that, in discussing protein requirements, plain figures of grams per kilogram will not suffice. We must take into consideration the biological value, as such, of the protein in the food.

I wish to make another point about the amino acid pattern. This pattern is at present a very fashionable concept, and people expect good results with a good amino acid pattern. I wonder whether, a few years hence, we shall not modify this concept. We already know, for instance, that Scrimshaw *et al.*,[5] by introducing supplements of amino acid to the so-called reference pattern, did not observe any improved results in the feeding experiments. Also, as reported recently, Gortner[6] has not been able to improve on the utilization of pure zein by adding any combination of amino acid to it. This leads us to a final remark: namely, to the concept of Mellander,[7] who considers (the evidence, for the time being, is not quite clear-cut) that perhaps not only the amino acid pattern, but the peptide pattern after digestion, must be considered. Some peptides might be absorbed and utilized as such, as keystones in the synthesis of protein.

As stated before, a protein food should be considered in its totality and not from the standpoint of its amino acid pattern alone.

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Nathan Talbot (*Massachusetts General Hospital, Boston, Mass.*): In considering the subject of protein nutrition it is of interest to make some simple calculations. For instance, we know that an average middlesized child weighing 30 kg. gains approximately 3 kg. year. It may be assumed in accordance with average adult body composition that approximately 70 per cent of this gain, or 2.1 kg., is made up of protoplasm. About 75 per cent of the protoplasm is water, and another 25 per cent is protein. In other words, the 30-kg. child ordinarily gains about 2.1 × 0.25, or 0.5 kg. of protein per year. This is the equivalent of 1.4 gm. of protein child day, or .05 gm./kg. of body weight/day. Added to the minimum maintenance figure of 0.3 gm./kg. mentioned by György, the result is the interestingly low value of 0.35 gm./kg./day. It is, of course, appreciated that this figure provides no leeway for inefficiency of utilization or for the fact that some proteins are incomplete.

Some perspective concerning the efficiency of protein utilization by the healthy, well-fed human may be had by considering the infant during the first 6 months of life, at which period he is growing faster than at any other time in life. Ordinarily the child approximately doubles his birth weight during this interval. In doing so he is increasing his body protein at the rate of about 1 gm./kg. of body weight day. If we add to this amount 0.3 gm. for maintenance, we obtain a minimum need value of 1.3 gm. kg. day. If he derives all of his nutrition from his mother's milk, his average daily protein intake will amount to about 2 gm. kg. day, a value that is less than twice the maintenance-plus-growth needs calculated above. It seems unlikely that the human race could have survived all the rigors of the evolutionary past if this had been an inadequate protein allowance.

A factor of considerable importance in the foregoing connections is caloric balance. As pointed out by Talbot *et al.*,[1] increasing the caloric intake from inadequate (1000 cal. $m.^2$ day) to adequate levels (1600 to 1800 cal. $m.^2$ day) while the child constantly received 2 gm. of protein kg. day caused the nitrogen balance of this 16-kg. hypocaloric dwarf to change from a level of approximately zero to a level of +1.5 gm. day. Doubling the protein intake while calories were maintained at the same level failed to cause a sustained increase in the nitrogen balance.

These observations would appear to have a possible bearing on the first three papers presented in this monograph. Unless I am mistaken, in the studies reported therein, the tendency was to increase the total caloric intake and the protein intake simultaneously. If this were so, one wonders whether the differences in nitrogen balance that were attributed to differences in protein intake might not have been due in large part to changes in caloric balance.

Finally it might be well to recollect that the use of animal instead of vegetable-fruit nutrients greatly increases the costs of feeding expressed in terms of acres of land and man-hours of farm labor. While this fact may be of little moment to people who can afford and can readily obtain whatever they desire, it is of the greatest importance to people whose survival and health depend upon the wise and most economical utilization of every resource. Accordingly, I should like to join György in expressing the hope that there will be further discussions of the true, basic protein requirements of human individuals of various ages.

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Harold D. Lynch (*Evansville, Indiana*): My concern here is with protein nutrition from the viewpoint of the private practitioner.

Hypoproteinosis

A few years ago the author and his co-worker, W. D. Snively, Jr., called attention to the prevalence of poor nutrition in the United States in an article on hypoproteinosis of childhood.[1] A group of symptoms common among children was described for the first time as a syndrome due to a low-protein diet.

Symptoms. Briefly reviewed, this syndrome embraces a group of symptoms heard commonly in the office practice of pediatricians. Starting with, "My child won't eat," or "He is not gaining weight," the list continues with such gastrointestinal symptoms as vomiting, diarrhea, and constipation. It includes peevishness, pallor, frequent infections, and dental caries, sometimes rampant.

These symptoms are consistently associated with a characteristic dietary history. The diet is predominantly liquid, consisting largely of beverages of all sorts, including juices and milk, the latter supplying an abundance of minerals. The vitamin supplement is usually more than sufficient. The solid foods are almost exclusively of the sweet variety. The solid protein foods are slighted.

Analysis shows that such a diet, despite generous quantities of milk, provides less than 15 per cent of its total calories from protein. This is a low-protein diet, and it does not support good nutrition. It should be pointed out that these diets are inadequate only in protein, and that the syndrome produced is not complicated by other dietary deficiences, as is the case in malnutrition in other parts of the world.

Unfortunately, there are no laboratory tests to aid in the diagnosis of a mild or even a moderate degree of protein deficiency.

Treatment. Gratifying relief from this syndrome is obtained when the level of protein calories is increased and maintained above 15 per cent. It will be shown later, however, that this relief is not accomplished easily.

If this condition is so prevalent, why is it not recognized and treated or prevented?

Unspectacular and unrecognized. The majority of practicing physicians have no deep interest in nutrition, and for good reasons. Medical schools in the United States, with one or two exceptions, do not have full-fledged departments of nutrition. Most of us have had no formal training in this really basic subject, which is so important to child health and to preventive medicine. We are thoroughly instructed about the conquest of diseases by means of drugs and/or surgery. However, we have had to learn about health and about nutrition and about the individual we are treating by practical experience alone. The medical practitioner, busy with the more financially rewarding struggle with diseases, has little time to educate himself in matters of nutrition.

Furthermore, parents do not, as a rule, associate their children's poor health with poor diet, so they seldom consult the doctor about nutritional problems after the infant feeding period. On the rare occasions when they do so, the parents are likely to present the child to the physician with a single complaint and ask for a specific cure, such as a tonic to make him eat or some medicine for his bowels. Often they get the advice that the child will outgrow whatever ails him.

The symptoms of faulty nutrition are so unspectacular that they are looked upon as normal isolated episodes in the child's development. No doubt this is the chief reason why, heretofore, these symptoms have not been considered as a related group with a single cause.

However, even when the relationship between symptoms and poor diet is recognized, the cure for hypoproteinosis may be difficult, because it cannot be effected by the simple advice to give the child more protein; it takes more than advice. The effective treatment of the child requires a revision of the feeding behavior of the mother and family. Parents must be taught how to persuade their child to take more protein. This requires the changing of adult attitudes and beliefs about foods. This is a task for the physician that is time-consuming as well as unremunerative.

Obviously, a disease that is seldom recognized and is troublesome to treat produces little incentive for an investigative crusade.

Causes of Low-Protein Feeding

In many parts of the world the reason for the child's failure to get enough protein is simple protein foods are not available. This is true only to a limited extent in the United States, where there are complicating psychological forces that often interfere with the child's best interests. The feeding of the children gets entangled in the social and emotional life of the whole family.

Parents must live with child feeding day in and day out, year after year. It is hard to sustain interest in any such prolonged task. Furthermore, parents are not prepared to deal with a problem that has so much to do with their own behavior.

One might ask, what effect does family life have on the child's protein intake? The answer is, of course, a great deal. Here is what the clinician sees:

During the first year, a large gain of approximately 15 lb. provides the infant with an eager willingness to take food.

By the beginning of the second year the child's rapid rate of growth has moderated and it soon reaches its low point. The sudden deflection in rate of growth has a profound influence on the appetite. Along with a physiological disinterest in food, the child's development in other directions begins to have disturbing effects on his behavior. He is becoming a human being and, as such, he becomes assertive and opinionated about all things, especially about foods.

The mother's instincts and emotions do not permit an easy adjustment to this turn of events. The close association between feeding her child and loving him sometimes prevents any adjustment at all.

During the first year the mother could give what the doctor prescribed in measured amounts that were large enough to satisfy her urge to feed the child.

Now suddenly this new element, the human element, makes it difficult to feed certain foods in any amount. However, a good mother "gets food into her child," so she gives him what she can, whenever she can.

Beverages, including milk and juices, can be poured in fairly easily. Feeding the solid protein foods requires the child's cooperation; he must chew them. Also, protein foods are not sweet and tempting; therefore they do not fit into the feeding routine with ease. On the other hand, desserts and sweet, palatable between-meal snacks readily win the child's approval in this interpersonal enterprise. Such a diet obviously is long on carbohydrate and short on protein. This is the diet of a multitude of American children.

It is a disconcerting fact that parents do not know about protein and do not realize its importance for their children. The term is seldom heard in the language of child feeding; protein is thought of only in connection with pet animals and livestock. However, parents all know about calcium for their children and, although a child's feeding behavior may be obstructive, the mother at least "gets his milk (with its calcium) into him." This often is a hollow victory.

Milk

This is a good place in which to point out how a misguided enthusiasm for milk contributes to the problem of giving the toddler and preschool child enough protein.

No one can deny milk its place as a source of important nutrients for mankind. When used with restraint or to make cheese, it is a valuable food for children. However, its almost fanatic use as a beverage has added more complications than benefits to child nutrition.

The slogan "one quart of milk daily" is taken literally to mean that this amount should be given to all children, regardless of age or capacity. There is no long-term clinical evidence to sustain this dictum.

It is not clear what diseases or symptoms the prolonged use of large quantities of calcium-rich milk will prevent, but certainly it often prevents the child's getting enough protein. It is almost impossible for the toddler and preschool child to have a satisfactory feeding program when his milk intake exceeds one pint daily.

Under home conditions, the child who takes large quantities of milk develops an irresistible appetite for desserts and sweets. He seldom likes the protein foods.

The Three-Meal Routine

Another obstacle in providing the child with enough protein is the American tradition of the three-meal routine. Throughout his long span of development the child is at cross purposes with this tradition.

The preschool child does not need three large meals daily, and he will not take them; usually he will not eat more than two fairly good meals. Of course, he cannot be expected to eat one good meal if each succeeding one is spoiled with between-meal snacks of beverages or sweets.

The small child in our civilization is not allowed to experience one of life's basic pleasures: that of getting hungry enough to enjoy the protein foods he needs for good health.

Mothers should be taught that hunger is not a thing to be dreaded, but something to encourage.

This is the basis for a few simple rules the physician can suggest to parents that will help them provide a program for the child's best interest without martyrdom for the rest of the family.

The period from 1 to 6 in a child's life is a crucial time physically, psychologically, and nutritionally. This is the time when his rate of growth and his appetite are at low ebb. Strangely enough, according to Brock,[2] and to Stearns' paper elsewhere in this monograph, the child seems to need the largest amount of protein per 100 calories during this period. Unfortunately, however, under conditions of our civilization, his protein intake is then likely to be lowest.

The Older Child and the Adolescent

Many forces contribute to supplying the older child with a subminimal amount of protein calories. Here again, the child's interests clash with the traditional American three-meal routine, but in a different way than when he was younger. Now three meals do not provide enough food.

The older child needs extra food between meals especially after school and at bedtime. These snacks should be considered extra meals—informal ones to be sure, but nevertheless important to the ravenously hungry school child. The same amount of care is needed in the selection of these snack items as in the selection of foods at mealtime. This consideration is rarely given. Failure to provide good foods, that is, those that contain proteins, at snack time nullifies the most carefully laid plans for a good diet.

The snack foods usually consist of such inappropriate items as candy, leftover desserts, crackers, cookies, potato chips, and popcorn; foods that taste good, that are put up in attractive packages, that require no refrigeration or preparation. These carbohydrate foods make up a disturbing proportion of the older child's diet. As the carbohydrates increase, the protein foods are correspondingly slighted. The resultant low-protein diet is consumed at a time when rapid body growth is literally begging for the building blocks of protein at a time when the defense mechanism against infections needs to be reinforced with protein.

Conclusion

The complexities of our society, rather than economics, have conspired to make it difficult for the child to obtain enough protein. Pressures on the protein level of the child's diet recur regularly throughout his many years of growth and development.

Feeding the child, which is a long task, in reality becomes an uphill struggle to provide him with enough protein.

Good child nutrition in our civilization requires the development of an enlightened philosophy of nutrition in order to apply the science of nutrition. This will require the combined efforts of the biochemist, the clinician, and the psychiatrist.

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Jack Metcoff (*Michael Reese Hospital, Chicago, Ill*): The difficulties with evaluating growth as a measure of protein requirement are, I think, quite obvious, as Genevieve Stearns has indicated. With growth, the major variable component added to the body is water, which accounts for 60 to 70 per cent of total body mass. During the first year of life there is an absolute increase of body water of about threefold to fourfold, but there is a relative decrease of water referable to body solids. Weight change is the resultant of these factors. The protein requirement with growth, particularly during the first year of life, diminishes relative to increasing mass and surface area.

In an investigation by James Gamble, a decreasing rate of weight gain and nitrogen retention is noted during the first 9 months of life.[1] This work is based upon studies of apposition of body mass determined by direct analysis of body composition at several ages made by Camerer over 50 years ago.[2-4]

The problems presented by balance measurements or creatinine excretion as indices of changing body mass are difficult to resolve. Creatinine excretion is dependent upon the rate of methylation of methyl glycosamine, an hepatic function, and upon the rate of renal excretion of creatinine. As clearance increases with increasing age during the first year of life, removal of creatinine becomes proportionately greater. This is somewhat independent of body mass, but it does bear a better relationship to body surface, since the young infant is more of a sphere than he is an elongated cylinder, in contrast to the older youngster or adult. Balance studies are complicated by the fact that if they cover too long a period some kind of systematic error attends them. For example, if one were to add up all of the salt that is known to be retained or apparently retained during the course of balance measurements over a period of several years, as Gamble has pointed out, the body would look very much like Lot's wife. If, on the other hand, short periods of balance of 3 to 5 days are carried out at repeated intervals, it is probably not fair to extrapolate these over a year's period of time, particularly when there is rapid change in tissue composition. Balance measurements, as we know, also are plagued by extensive skin losses, including skin losses of nitrogen. At low intakes the skin losses comprise relatively greater proportions of the total balance.

Finally, as Talbot has mentioned, there is a striking difference between optimum protein in relation to continuing growth and protein requirements necessary to sustain and/or to prevent catabolism of tissue.

For example, in a young infant whose intake was limited to an amount of glucose equivalent to that commonly used to provide calories during periods of parenteral fluid therapy, the ratio of loss of potassium to nitrogen was found to by highly variable, although theoretically it should have been consistently at about 3.0. At the beginning of the fasting period the loss of potassium was considerably in excess of the loss of nitrogen, and with cessation of fasting the gain of potassium was in excess of that of nitrogen. There appeared to be a progressive decline in the K N ratios, as derived from the balance measurements during fasting. In contrast, during control periods the K N ratios varied from 3 to 6 in different periods, but were fairly consistent in each period. Since other components of protoplasm may vary independently of nitrogen, and since nitrogen retention may represent growth of such bodily components as hair, bone, muscle, organs, and skin, in varying degrees, this measure of growth is not completely satisfactory. Nitrogen balances are often in excess of apparent amounts of actual protoplasm formed, as Talbot has indicated.

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J. Roswell Gallagher (*The Adolescent Unit, The Children's Hospital, Boston, Mass.*): There are three general aspects of Joseph A. Johnston's excellent paper, upon which I shall comment briefly. The first has to do with what at times seems to be the preoccupation of both the physician and the public with greater-than-average weight; the second, with a failure to remember one's patient's needs for total development and a tendency, instead, to focus upon some abnormality; and the last, with the importance of determining the effect of any recommendation upon healthfulness and effectiveness in adult life, rather than upon these matters in childhood or in adolescence alone. One's belief regarding the protein intake desirable for adolescents relates to each of these.

To take the first of these points: our considerable interest in those whom we believe to be overweight. In our clinic it is common to find that an adolescent who has been referred to us for treatment of excess weight is already taking in fewer calories and much less protein than his thinner friend. Our concern with these young people has been threefold: first, to determine how much of their greater than average weight is in reality excess fat; second, to determine to what extent exercise and activities are factors; and, finally to be sure that their protein intakes are kept at levels which, in the face of lower calories, will adequately supply their needs. The routine recommendation of caloric reduction without concomitant consideration of an adolescent's protein needs may result in a less than optimal diet.

A girl recently seen in our clinic illustrates this point: at 16 years, her height was 69 inches and her weight 190 pounds. She had been told that she must lose at least 50 lb., and a 1000-cal. diet had been suggested. No inquiry had been made into her degree of activity, which was unusually great, nor had an estimate been made of her degree of fatness, which was quite low. An effort to help her to accept herself, the physician's avoidance of appearing dissatisfied with her, and some increase in protein and calories would seem more appropriate for this very large, very active, but not very fat young person.

It is fair to say that none of us know, and that most of us are confused about, the optimal protein intake for the adolescent. Recently in a preliminary study of 20 boys, one half of whom were quite fat, we were struck by the fact that the nonobese group took in from 3300 to 5500 calories, whereas the intakes of their fat friends were from 2600 to 3500 calories, and that the protein intake of the former ranged from 120 to 190 gm., as against 30 to 140 gm. for the obese boys. The free-choice protein intake of the thinner boys may have been unnecessarily high. One wonders if the adolescent should not have more protein than is often recommended in diets given those who are considered overweight. It would certainly seem wise always to remember that it may have been erroneously assumed that an overweight adolescent is consuming an adequate amount of protein.

My second comment, that at times we focus upon an abnormality and ignore the needs and characteristics of our patients and their age group, develops from my first point. Adolescence is not only a time of rapid physical growth; it is also normally a time of very strenuous activity, activity that is relentlessly pursued in efforts to gain the recognition and applause of the adolescent's associates. To do things strenuously is normal for them; moderation is unusual.

For us to neglect to foster activity, to forget its demands and its significance, or to ignore the implications of inactivity in the adolescent and to focus our attention on the fatness or thinness is to forget what sort of a person our adolescent is. By and large, when one is dealing with an adolescent, it would seem better, in the face of obesity, for instance, to concentrate on seeing that activity is high and that protein intake is reasonable, rather than to think predominantly of decreasing calories.

Finally, another remark upon one of the points that Johnston has made: in determining the optimal levels of protein intake for adolescents, we need to think of more than freedom from illness, of more than what seems to be adequate for body growth. If we are to be sure of the validity of our opinions regarding their protein requirements, we should know how these adolescents develop over a period of several years in short, what sort of adults they become. My chief dissatisfaction is with those whose very positive opinions regarding such matters as optimal protein intake for adolescents are based upon their observations of these young people at a single point of time or over a very limited number of years. Basically, our efforts to help the adolescent should be to help him or her to become a better adult. To know whether this or that is good for him, we need to follow his health status and his intellectual status into adult life. Certainly we are interested in more than what seems to produce, or to occur in, a healthy adolescent. We really want to know what it is that is most likely to produce an effective and healthy adult.

THE ASSESSMENT OF PROTEIN NUTRITION IN AGED MAN

By Donald M. Watkin

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Many studies of protein nutrition in aged human subjects have been confined to dietary surveys, clinical appraisals, collections of data on total serum proteins and hemoglobin, and studies of nitrogen balance. These evaluations have led to confusing and often conflicting opinions on the protein needs of old people. For example, Kountz, Hoffstatter, and Ackermann[1] interpret data from balance studies in old men to indicate an average minimum requirement of 0.7 gm./kg./day. They recommend as a safe allowance, in view of much higher minimum requirements among some of their subjects, an intake of 1.4 gm./kg./day. In contrast, Schulze,[2] from his own studies of nitrogen balance and metabolic rate in elderly healthy volunteers and a comprehensive review of the literature, concludes that, if anything, the minimum requirement for old subjects is actually less than for young.

Lack of agreement among different workers may be attributed to four factors:

First, agewise changes per unit of time are small. An example is the small change with age in total body water measured by antipyrine. The average values in 93 subjects studied by Shock, Watkin, and Yiengst[3] fell from 34.5 l. in the third- and fourth-decade groups to 29.7 l. in the ninth-decade group, a decrease of less than 100 ml./year.

Second, interindividual differences among subjects are large. An example of this is presented in FIGURE 1, where one 78-year-old subject has an antipyrine space comparable to several found in the 35- to 40-year-old range. The age regression fitted to the points by the method of least squares is statistically significant, but only because of the large number of subjects involved. In view of this great interindividual variation, it is obvious that no valid conclusions could be drawn from a smaller series. Marked variability among aged subjects is not unexpected when the infinite variations of their medical, nutritional, social, and economic backgrounds are borne in mind.

Third, the intraindividual biological variability of any parameter under study may be appreciable. This problem with respect to serum beta lipoproteins and total cholesterol has been discussed by Watkin, Lawry, Mann, and Halperin.[4] FIGURE 2 shows the wide fluctuations observed in a single 78-year-old subject from this study. The authors constructed a table showing that single determinations were highly unreliable in estimating average values of beta lipoprotein levels. As many as 21 determinations may be needed in a single individual to obtain with reasonable accuracy an average value representative of the true level. Other studies showing the existence of long-term trends in balance study data[5] further emphasize the importance of intraindividual variability in interpreting any experiment in the aged.

Finally, experimental conditions differ from study to study among lab-

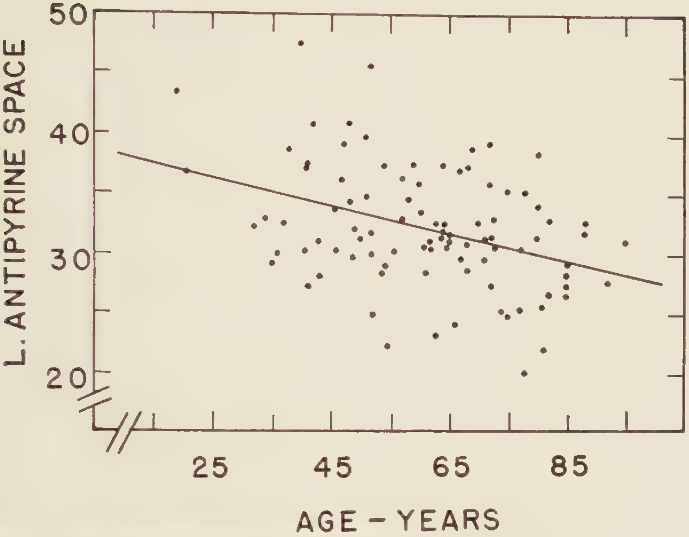

Figure 1. Liters of antipyrine space in relation to age in 93 healthy male subjects.

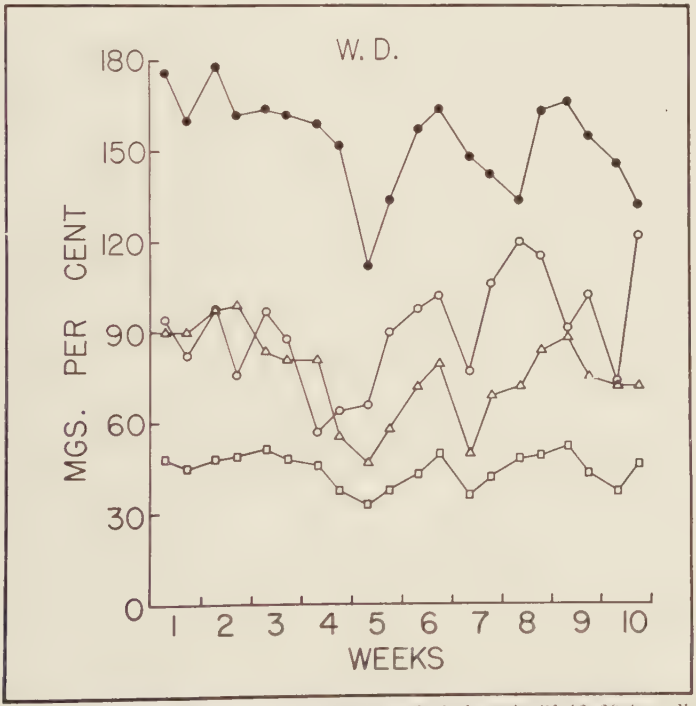

Figure 2. Variations in total cholesterol (closed circles), in Sf 12–20 beta lipoproteins (triangles), Sf 21–35 beta lipoproteins (squares), and Sf 35–100 beta lipoproteins (open circles) in a 78-year-old man. Reproduced by permission from *The Journal of Clinical Investigation.*[4]

oratories and even within the same laboratory. Temporal, economic, and clinical factors may dictate short studies of a limited number of parameters.[6] Long control periods, essential to establish inter- and intraindividual variability,[7] are often omitted. Even under ideal conditions, unwitting prejudice may creep into an experimental design. It is not impossible through the biased selection of experimental conditions to come to opposite conclusions in two studies conducted at different times in the same laboratory and even within the same individual.

In view of the difficulty in quantitating exactly the various parameters in the aged, it is not surprising that the majority of authors have concluded that differences in protein requirements between healthy young and healthy old individuals are either nonexistent or too small to be of practical import in the United States today.[5, 8-10] Adherence to standard recommendations on protein allowance such as those of the National Research Council[11] (1 gm. kg. day) seems entirely justified in view of available information.

Despite this compromise solution dictated by lack of detailed knowledge, there is no lack of academic interest in seeking information that is more intellectually satisfying. Broader applications of conventional methods and the introduction of advanced technological procedures now enable clinical investigators to examine with more precision subtle differences in protein nutrition that may exist between young and old.

An excellent example of the new approach is the attention now being focused on the amino acid composition as well as the gross amount of dietary protein. Mertz *et al.*[12] showed by determination of the amino acid composition of self-selected diets that older women who ingested acceptable amounts of protein nitrogen were in negative nitrogen balance because of a limited intake of methionine. Tuttle *et al.*[13] found that men over 50, fed quantities of L-amino acids sufficient to maintain nitrogen balance in young adults and containing nitrogen in excess of that which the older men needed for equilibrium on a regular diet, were consistently in negative nitrogen balance. These investigators infer from their data that old men have a quantitatively increased requirement for one or more amino acids. In considering these experiments it must be recalled that investigations of amino acid mixtures differ markedly from studies with whole proteins. More calories are required. The timing of administration of the mixture is critical. The source of nonessential nitrogen has a marked effect on the final balance. Further studies in aged man of mixtures of individual L-amino acids are needed to demonstrate the dimensions of this problem.

A second example of a new approach is the attention focused in recent years on studies of total body composition. Shock, Watkin, and Yiengst[3] have studied the relation of total body water measured with antipyrine to basal metabolic rate and oxygen consumption, as indicated in FIGURE 3. They find that total body water per square meter of body surface decreases with age, that basal metabolic rate expressed as calories per square meter of body surface also decreases with age, but that basal metabolic rate expressed as ml. oxygen consumed per liter of antipyrine space does not decrease with age. In other words, there is no decrease with age in oxygen consumption per unit of metabolizing tissue. There is no change in the quality of the protoplasm with age, but only in the amount of it. In view of these findings, it may be appropriate to suggest that protein requirements are related directly to protoplasmic mass and only indirectly to basal calories per unit of surface area.

A third approach has been the investigation of tissue composition. Yiengst, Barrows, and Shock,[15] studying differences in composition between muscle of 12- and 24-month-old rats, observed a significant increase in extracellular space but a decrease with age in intracellular water, potassium, phosphorus, and nitrogen. These changes were not associated with changes in nitrogen/phosphorus, potassium nitrogen, or potassium phosphorus ratios, and they therefore suggested a loss or atrophy of cells and their replacement by extracellular fluid. Similar changes, if also observed in man, would provide support for the conclusions resulting from studies of total body composition. The role of protein nutrition in the prevention of this apparent cellular loss or atrophy with advancing age has yet to be investigated.

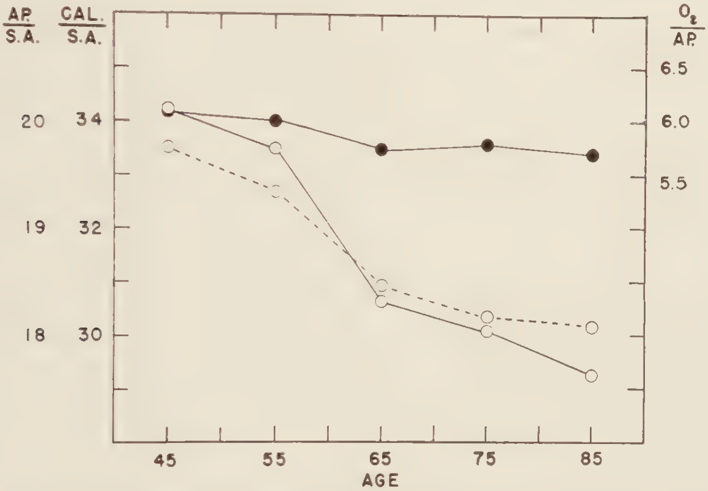

FIGURE 3. Age in relation to antipyrine space (liters per square meter of body surface are shown by the open circles connected by the broken line), to basal metabolic rate (calories per square meter of body surface are shown by the open circles connected by the solid line) and oxygen consumption per unit of functioning protoplasm (milliliters of oxygen per liter of antipyrine space are shown by the closed circles). Reproduced by permission from the National Vitamin Foundation.[14]

A fourth approach to the evaluation of protein nutrition in the aged has been the electrophoretic examination of serum proteins in young and old men. Karel, Wilder, and Beber[16] have reviewed the studies of Rafsky *et al.*[17] and have presented their own findings in 21 elderly and 9 young subjects. The mean values from this study appear in TABLE 1. A decrease in total protein and albumin and elevations in all the globulin components is obvious. A possible role of inadequate dietary protein in producing the differences between young and old is suggested by the studies of Watkin *et al.*,[18] in which the gradual elevation of total serum proteins and albumin was observed in old men forced to consume over 100 gm. of protein daily after existing for years on a presumably adequate institutional diet. Subtle changes in protein synthesis in the liver offer an alternative explanation.

A fifth approach is the use of the complete or total-balance study technique. Balance studies including an evaluation of potassium, calcium, phosphorus, sodium, and calories enable the investigator to assess changes in body composition with a precision impossible through the use of nitrogen balance alone. Watkin *et al.*[15] used the balance technique to evaluate the relative roles of protein, calories, and androgen administration in the nutrition of 8 elderly men. Extending over a 5-month period, this study consisted of a high dietary protein phase (106 gm./day) followed by a low-protein phase (52 gm./day). Each phase was divided into a control period, a period during which each subject received 25 mg. of stanolone, an androgenic steroid, daily, and a follow-up period. In each phase, one half of the patients received a diet of 1800 calories, and the remainder a diet of 2600 calories. Data obtained from a typical subject are presented graphically in FIGURES 4 and 5. The average balance data from the entire experiment are presented in TABLE 2. An examination of the nitrogen data shows the marked effect of dietary protein level when the 2 control periods are compared. The data also show greater nitrogen retention in response to hormone administration on a high than on a low protein intake as well as a distinct nitrogen-retaining effect of the hormone itself.

TABLE 1

AVERAGE VALUES OBTAINED FROM THE ELECTROPHORETIC PATTERNS OF SERUM PROTEINS IN 21 ELDERLY PERSONS AND 9 YOUNG ADULTS*

	Per cent of total protein		Grams	
	Young	Aged	Young	Aged
Total protein	100	100	6.88	6.08
Albumin/globulin ratio	—	—	1.44	0.88
Albumin	59.14	46.07	4.11	2.80
Globulins	40.86	53.93	2.77	3.28
Alpha 1	5.04	6.30	0.35	0.38
Alpha 2	10.04	12.69	0.71	0.77
Beta	13.43	17.33	0.93	1.05
Gamma 1	2.68	3.55	0.18	0.20
Gamma total	12.34	16.17	0.84	1.07

* Adapted from Karel, Wilder, and Beber.[16]

The balance data have been subjected to an analysis of variance to assess the importance of calories as well as protein on the elements under study. The results of this analysis appear in TABLE 3. They suggest that the difference between the caloric levels studied (800 cal./day) has no influence on the nitrogen balance under the conditions of this experiment. The effect of dietary protein level, however, was highly significant. There was no influence of calorie-protein interaction. Individual variation was significant for nitrogen; that is, differences among individuals were greater than period-to-period differences within individuals. Individual-by-protein interaction was without influence on nitrogen balance.

To assess the role of dietary protein level on the metabolic response to hor-

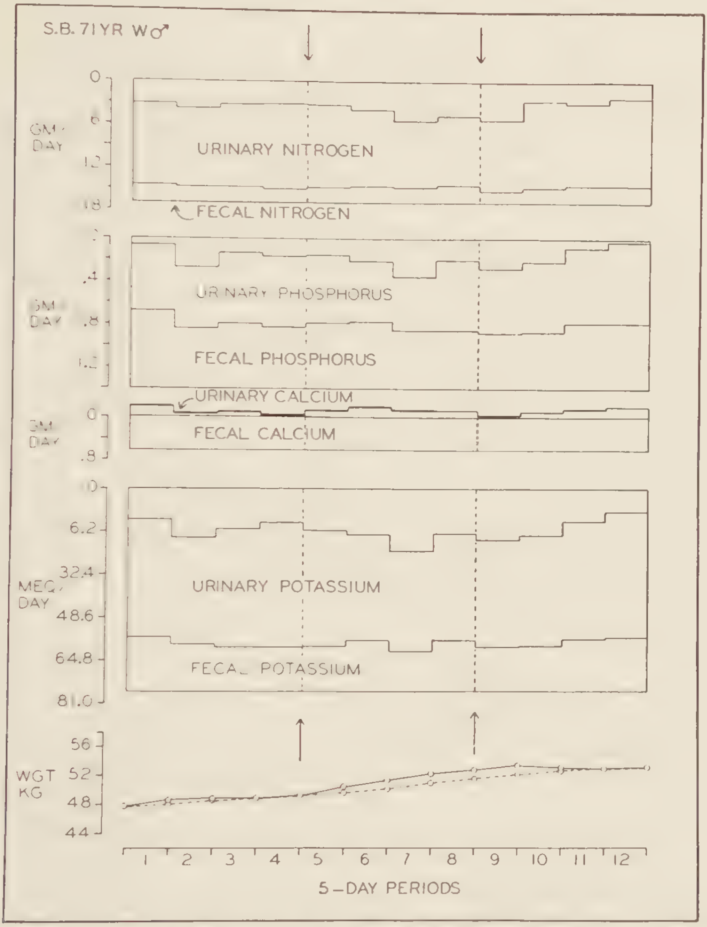

Figure 4. Records of a 71-year old man. The effect of stanolone administration (25 mg./day) on nitrogen, phosphorus, calcium, and potassium balances and on body weight while the subject was consuming a diet high in protein and in calories. The balance charts are constructed by plotting the intake downward from the zero line and then, plotting upward from the intake line, first fecal and, second, urinary excretion. If the sum total of excretion coincides with the zero line, equilibrium is indicated; if it extends above, the balance is negative; and if it lies below, the balance is positive. The ordinates of the nitrogen, phosphorus, calcium, and potassium charts are so selected that equal heights represent the approximate ratios at which these elements exist in normal protoplasm and bone. Stanolone was administered during the periods between the arrows. The circles connected by solid lines indicate the observed body weight; the circles connected by the broken lines indicate the theoretical weight calculated from the nitrogen and potassium balances.

mone administration, a modified *t* test analysis was performed on the data. In TABLE 4, the values for *t* obtained from the comparison of pretreatment and treatment periods appear in the first column; from the comparison of high and low protein regimens, in the second column; and from the interaction of protein and hormone therapy, in the third column. A significant effect of hormone therapy on nitrogen balance at both levels of protein intake is shown by the *t* values in the first column. The significant effect of dietary protein level on nitrogen balance is shown by *t* values in the second column. The lack of any significant hormone-protein interaction is shown in the third column.

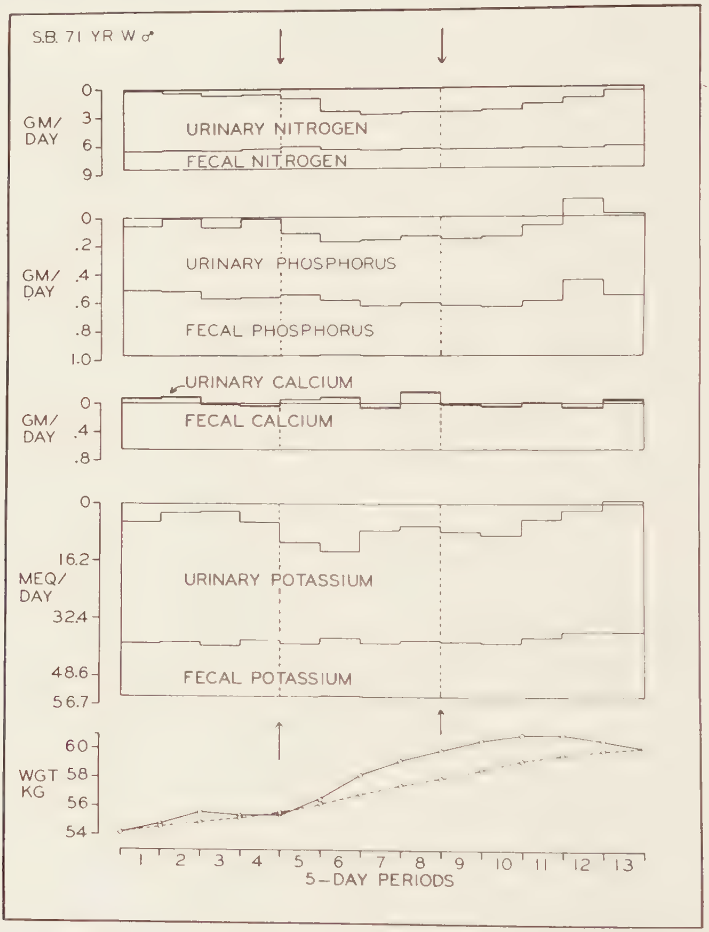

FIGURE 5. Records of a 71-year-old man. The effect of stanolone administration (25 mg./day) on nitrogen, phosphorus, calcium, and potassium balances and on body weight while the subject was consuming a diet low in proteins and high in calories.

From these studies it is apparent that a high-protein diet is a greater stimulus

TABLE 2

AVERAGE BALANCES OF NITROGEN, POTASSIUM, PHOSPHORUS, AND CALCIUM IN 8 ELDERLY MEN DURING PRETREATMENT (C), TREATMENT (E), AND POSTTREATMENT (F) PERIODS ON HIGH (H) AND LOW (L) PROTEIN DIETS; MEANS AND STANDARD ERRORS OF THE CHANGES WITHIN INDIVIDUALS IN NITROGEN, POTASSIUM, PHOSPHORUS, AND CALCIUM BALANCES BETWEEN TREATMENT AND PRETREATMENT (E-C), AND POSTTREATMENT AND TREATMENT PERIODS (F-E), AND HIGH AND LOW PROTEIN DIETS (H-L)*

Balance	Diet	C Control	E Experimental	E-C Experimental-control	F Posttreatment	F-E Posttreatment-experimental
Nitrogen, gm./day	H	2.92	4.55	+1.63 ± .20†	2.94	−1.61 ± .28†
	L	0.64	1.88	+1.24 ± .18†	1.14	−.74 ± .16‡
	(H-L)	+2.28 ± .10†	+2.67 ± .21†	—	+1.80 ± .19†	—
Potassium, mEq./day	H	8.6	16.7	+8.1 ± 1.4†	9.7	−7.0 ± 1.7‡
	L	2.2	7.4	+5.2 ± .6†	2.3	−5.1 ± 1.0‡
	(H-L)	+6.48 ± .59†	+9.22 ± 1.25†	—	+7.38 ± .77†	—
Phosphorus, mg./day	H	178	300	+122.4 ± 27.7‡	169	−131.5 ± 32.9‡
	L	65	130	+65.1 ± 11.7‡	76	−54.1 ± 11.4‡
	(H-L)	+112.4 ± 13.1†	+170.6 ± 25.7†	—	+92.2 ± 17.6‡	—
Calcium, mg./day	H	−26.9	−32.0	−5.1 ± 22.7	−61.5	−29.5 ± 18.6
	L	11.2	−23.6	−34.9 ± 15.2	+5.6	+29.2 ± 10.9
	(H-L)	−38.1 ± 28.1	−8.4 ± 15.4	—	−67.1 ± 15.1‡	—

* From Watkin, Parsons, Yiengst, and Shock.[18]

† Significant $P < .001$.

‡ Significant $P < .01$.

to nitrogen retention in the aged than either added calories or an androgenic hormone. Although the retention of nitrogen during androgen administration was slightly higher on the high- than on the low-protein diet, it has not been possible to demonstrate a statistically significant hormone-protein interaction.

In another series of complete balance studies, Watkin and Steinfeld[19] have examined the effect of caloric hyperalimentation by fat emulsion administered intravenously in patients of various ages with and without malignant disease. During the control phases, elderly subjects with malignancies were in negative calorie balance despite positive balances of nitrogen (TABLE 5), potassium, and phosphorus. A 900-cal. daily intravenous supplement resulted in more positive nitrogen balance but no change in the negative calorie balance. In other words, the patients were expending calories, largely from fat, while gaining nitrogen. Their caloric expenditure increased in proportion to calorie intake, leaving them in negative calorie balance despite caloric hyperalimentation. A change in body composition characterized by a wasting of fat and retention of nitrogen can be inferred from the data. This study demonstrates the worth of examining multiple parameters in evaluating the problem of protein nutrition in aged man.

TABLE 3

THE LEVELS OF SIGNIFICANCE OF THE INFLUENCE OF CALORIES, PROTEIN, CALORIE BY PROTEIN INTERACTION, INDIVIDUAL VARIATION AND INDIVIDUAL BY PROTEIN INTERACTION ON THE BALANCES OF NITROGEN, POTASSIUM, PHOSPHORUS, AND CALCIUM DURING PRETREATMENT (C), TREATMENT (E), AND POSTTREATMENT (F) PERIODS

	C				E				F			
	N	K	P	Ca	N	K	P	Ca	N	K	P	Ca
Calories	n	n	.05	.05	n	n	n	n	n	n	.2	.2
Protein	.001	.001	.001	n	.001	.001	.01	n	.001	.001	.01	.01
Calories by protein	n	n	n	n	n	n	n	n	n	n	n	n
Individual	.05	.01	n	n	.05	.001	n	n	.001	.1	.01	n
Individual by protein	n	n	n	n	n	.2	n	n	n	n	n	n

n = P > .2.

TABLE 4

VALUES FOR "t" OBTAINED FROM A COMPARISON OF PRETREATMENT (C) AND TREATMENT (E) PERIODS, A COMPARISON OF HIGH AND LOW PROTEIN REGIMENS AND FROM THE INTERACTION OF PROTEIN AND HORMONE THERAPY

	C versus E	High versus low	Protein versus hormone
Nitrogen	−3.568	8.488	−0.537
Potassium	−2.706	5.024	−0.700
Phosphorus	−2.044	2.804	−0.551
Calcium	0.562	0.474	0.363

A sixth example of a new approach to the study of protein nutrition in the aged is the use of isotopically labeled proteins in the investigation of protein absorption and its synthesis and degradation in man. Chinn, Lavick, and

Cameron[20] concluded that I^{131}-labeled albumin mixed with gelatin was digested and absorbed equally as well in the aged as in the young. I^{131}-labeled human serum albumin has also been used by Steinfeld[21] to demonstrate in albumin turnover studies a depressed albumin synthesis in elderly patients with malignant disease. Such studies have yet to be done in large numbers of healthy aged subjects.

A final example of a new approach is a study by Silverstone, Watkin, and Shock,[22] who measured nitrogen, phosphorus, and potassium balances, discrete renal functions, blood chemistries, and hematologic values in young and aged groups of healthy men on moderate, low, and high protein diets. Analysis of the cumulative nitrogen balance data revealed no statistically significant differences between the abilities of the young and old groups to adapt to sudden decreases in protein intake. The old group did, however, show a transiently greater positive nitrogen balance than did the young when shifted from the low to the high protein diet. This was accompanied by an increase in blood nonprotein nitrogen that was more marked in the old. The change from a low to a high protein diet was accompanied by an increase in glomerular filtration rate and renal plasma flow but no significant change in Tm_{PAH} in both young and old groups. The transient elevation in nonprotein nitrogen and the greater positive nitrogen balances observed in the older group correlate well with the lower glomerular filtration, in absolute terms, characteristic[23] of aged man.

TABLE 5

CALORIE AND NITROGEN BALANCES IN A 70-YEAR-OLD MAN WITH CARCINOMA OF THE KIDNEY BEFORE, DURING AND AFTER HYPERALIMENTATION WITH FAT EMULSION ADMINISTERED INTRAVENOUSLY

Six-day period	Calories per 24 hours			N in grams per 24 hours		
	Intake	Expenditure	Balance	Intake	Output	Balance
Control 1	2362	3374	−1012	13.54	12.94	+0.60
Control 2	2362	3508	−1146	13.54	12.28	+1.26
Control 3	2362	3196	−834	13.54	12.46	+1.08
Hyperal 1	3234	4324	−1090	13.54	10.93	+2.61
Hyperal 2	3289	4374	−1085	13.54	10.87	+2.67
Hyperal 3	3247	4350	−1103	13.54	11.87	+1.67
Hyperal 4	3269	4383	−1114	13.54	12.74	+0.80
Hyperal 5	3260	4670	−1410	13.54	12.34	+1.20
Hyperal 6	3283	3957	−674	13.54	12.83	+0.71
Follow 1	2362	4163	−1801	13.54	12.92	+0.62
Follow 2	2362	3912	−1550	13.54	13.41	+0.13
Follow 3	2362	3386	−1024	13.54	13.51	+0.03
Follow 4	2362	2858	−496	13.54	13.95	−0.41

Summary

No experiments have been performed that indicate conclusively either qualitative or quantitative changes with age in the protein needs of healthy adults. The assessment of protein nutrition in the aged is difficult because of the small magnitude of change per unit of age, the large variability both among individuals and within an individual, and the differences in experimental design and execution. For the moment, available evidence implies that an adequately balanced diet containing 1 gm. of protein per kg. satisfies the requirements of the healthy aged. Individual problems, however, call for special solutions tailored to the conditions at hand. Socioeconomic factors and the presence of disease have far more practical influence than age per se in determining the status of protein nutrition in the aged. Despite our lack of precise information on the interrelations of age and protein nutrition in man, broader application of conventional methods and the introduction of recently evolved techniques, several examples of which have been cited, offer new hope for more satisfactory answers to the academic problem.

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Discussion

Margaret A. Ohlson (*State University of Iowa, College of Medicine, Iowa City, Iowa*): Donald Watkin has emphasized the variability found in nutritional measurements of the aged, both between individuals and in one individual from time to time. Such variation is not surprising, since the aged present as wide a range of nutritional status as does any other age group; moreover, years of living have established habits of eating and physiological response that may be difficult to modify in any short experiment. Older people may achieve a satisfactory intake of nutrients in many ways. In a county home in Michigan housing many Scandinavians, potatoes contributed 15 to 20 per cent of the protein, and the residents were vigorous for their ages.

My colleagues and I have studied about 300 women, many of them for years. Of these, 28 have been studied many times, using the balance experiment and the technique of observing the self-selected diet and then varying the protein intake over a wide range. One group of subjects was in nitrogen equilibrium on intakes of 0.8 to 0.9 gm. kg. of protein. Additional protein caused retention only briefly; less protein resulted in loss of nitrogen unless calories were increased. However, these active women of 60 to 80 years found less protein and more than 1800 to 2100 calories unpalatable; they also found that such a dietary formula required special preparation, which made living in a family group a burden. Thus, although 0.8 to 0.9 gm. of protein per kilogram of weight may not have been a physiological minimum for this group of healthy, aging women, it represented a practical minimum.

The rest of the women of this series required more protein to maintain equilibrium, usually more than 1 gm. kg. of weight, and they retained protein when an extra amount was offered. In some cases retentions continued for months. Examination of the women themselves revealed that the first group were normal in weight, vigorous, and had few complaints of minor ill health. Dietary histories suggested that they had always eaten moderately, but well. The second group tended to be overweight and less vigorous, and had many minor complaints. Their dietary histories suggested irregular meals and little liking for the protective foods such as milk, fruits, and vegetables.

Many older individuals have learned to control the outward expression of emotions, but this does not preclude a physiological response. One well-poised 65-year-old woman who worked regularly as a janitress had been in nitrogen equilibrium for months on a daily intake of 1800 calories and 1 gm. of protein per kilogram. During 5 days she lost 20 grams of nitrogen after learning that her son had been injured in battle, but she did not change her food intake or show outward signs of stress. During the following week she retained an equal amount of nitrogen. This cycle of loss and retention was repeated 2 months later under similar circumstances. A vigorous 80-year-old became a shuffling old woman in 6 months after she gave up her large home and the care of several roomers to live in a small apartment adjoining her daughter's home, although the arrangement had been made with her full cooperation. The physiological responses of the aged are varied, but observations made over long periods can provide some understanding of the range of variation and some of the reasons for variation, the importance of which may then be evaluated.

FREDERIC D. ZEMAN (*The Home for Aged and Infirm Hebrews, New York, N. Y.*): Donald Watkin's excellent exposition of modern viewpoints and methods in studying the protein needs of elderly men and women has caused my thoughts to turn back to the spring of 1914, when I was completing my first year as a student at the College of Physicians and Surgeons of Columbia University, New York, N. Y. In the biochemistry laboratory I came to know Paul Howe, then an assistant professor in the department. The combination of Howe's honeyed words and my own innocence led to my becoming involved in a study of protein metabolism during and following fasting, in which I served both as a member of the team of investigators and as the subject. My fast lasted for 7 days, preceded by a period on a standard diet, followed by 4 days on a glucose-fat diet, and again by 4 days more of a standard diet. I do not propose to describe all the details, which are still vivid in my mind; I state merely that on the basis of these exhaustive and painful researches we were able to present 3 papers at the December 1914 meeting of the Federated Biological Societies in St. Louis, Mo.

When Fredrick J. Stare asked me to discuss this particular presentation, he could hardly have realized that he was evoking fond memories of my first scientific love. In the years that followed I became enamored in turn of pathological anatomy and of clinical medicine, particularly as related to the problems of old age. This personal background makes me appreciate all the more keenly Watkin's emphasis on interindividual and intraindividual differences and on the differing experimental conditions in various laboratories. I have emphasized elsewhere the difficulties in studying modifications of physiological response in the aging organism: "In setting up our plans of procedure we must study each individual with care to avoid including any with diseases that might alter the basic responses. We must exercise caution in utilizing old people classified only according to chronological age. We must not be deluded by apparent or outward health, since older people show a great contrast between diagnosable pathological lesions in their bodies and their actual functional capacity."[1] I like to describe the picture of disease in the aged as characterized by multiplicity, chronicity, and duplicity.

This illustrates very well the points that Donald Watkin and Margaret

Ohlson have emphasized in this field. The problem of working with the older people, whether one is studying them from the standpoint of physiology or pathology, is one of extremely complex or difficult nature in comparison with the work of our colleagues in pediatrics.

The need to plan investigations in such a way as to avoid deleterious effects on the subject would seem to be obvious enough, but one must also make every effort to control the effect of emotional stimuli upon the bodily reactions. I have often wondered what effects are produced in certain types of studies by the pain, fear, and anxiety caused by repeated venipunctures or by the discomfort of lying for hours with a catheter in the bladder. In old patients the unpleasant effects of experimental procedures are aggravated by the presence of long-standing psychoneuroses and by organic disease of the central nervous system, which may present no obvious symptoms.

The long-standing custom that one discussing a paper has not fulfilled his duty unless he picks upon some area, however trivial, that the essayist has either overlooked or treated inadequately is hardly possible in this case; nevertheless I now insert a small "but" by reminding the reader of the rapidly increasing body of knowledge on the pathology of protein utilization in the human, particularly as shown by the "inborn errors of metabolism." In the past few years I have become increasingly interested in these genetic disorders, particularly because so many of them do not manifest themselves until late in life, as do diabetes and gout, for example; furthermore, because these inborn errors of metabolism are not at all incompatible with a long and useful life. It seems to me that in these inborn errors of metabolism, in these peculiar breakdowns of bodily processes at some point in the processes of enzymatic degradation, we may see a very great possibility for future studies as our techniques for determining these substances become more accurate. I feel very strongly that a study of the genetic disorders may point the way to the imaginative investigator to seek new methods and new approaches that will lead to developments no less startling than those recorded since I first experienced my great hunger more than forty years ago.

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THE TREATMENT AND PREVENTION OF KWASHIORKOR IN FRENCH WEST AFRICA

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INTRODUCTION

In underdeveloped nations protein deficiency constitutes one of the most serious problems and has wide economic and social repercussions. Protein deficiency is especially serious during growth, and in children its worst effect is found in the guise of kwashiorkor, which ordinarily develops in the weeks following weaning.

In addition to the well-known and easily recognizable forms of the disease, there exist numerous other ill-defined forms that can lead, for example, to malfunction of the pancreas or liver and lay the foundation for more serious complaints in the future.

In adults and in children alike, lack of sufficient protein brings about lowered resistance, a phenomenon very common in Africa.

Treatment of advanced kwashiorkor by a high-protein diet gives excellent results, and the death rate from this disease, which was formerly 80 per cent, is now 20 per cent. However, on returning home from hospital, the child again becomes exposed to the deficient and unbalanced diet that originally caused malnutrition, and it is not unusual, one or two months later, to find the child suffering from a more severe case of the disease than when he was first hospitalized.

Therefore, the real treatment of kwashiorkor and of protein deficiency must be preventive and must be based on the two following essentials: on one hand, the education of the population, mothers in particular, and on the other hand, the production of protein nutrients of good biological value and at a price commensurate with the standard of living.

I shall begin by describing the treatment of the pure forms of kwashiorkor; this will enable me more easily to present the preventive treatment undertaken either with local products such as peanuts and fish flour or with isolated amino acids.

TREATMENT OF FRANK KWASHIORKOR

The treatment of kwashiorkor is now well established. Many writers have shown that a diet rich in proteins gives a favorable result. Skim milk, sometimes enriched by the addition of casein, has been used most frequently. However, in a very interesting study, Dean[1] has obtained excellent results by using soybeans.

Benefiting from earlier studies of the problem,[2-8, 11] and basing our work on personal experience in the treatment of 350 children suffering from kwashiorkor and hospitalized under our care over a period of 4 years, my associates and I have developed a method of treatment that seems to us to be effective.

I am concerned here only with cases of typical kwashiorkor; that is, kwashiorkor accompanied by clinical, biological, and histological symptoms that are now well known. Most common among these symptoms are edema, lesions of the skin and of the mucous membranes, changes in the hair, hypoproteinemia (less than 6 gm. per cent), the lowering of the amylase level, and fatty degeneration of the liver at biopsy.

These typical forms themselves differ in severity: one child will arrive in such critical condition as to die within the next few hours, while another obviously will not be so severely affected.

In any case, treatment must not be delayed, because deterioration may proceed rapidly. Children diagnosed "suspected kwashiorkor," controlled through consultation and not following the prescribed diet, can return 10 days or 2 weeks later looking the very picture of advanced malnutrition.

Choice of Proteins

Milk and even vegetable protein can give good results. Some workers enrich milk with casein in order to increase the protein level.

However, as the exocrine cells of the pancreas may have suffered early and serious damage, it seems better to use protein hydrolyzates that quickly make available to the body a greater quantity of amino acids and allow a functional recovery of the enzymatic system with much less delay.

In furtherance of this aim, we used a protein hydrolyzate prepared by a milk-chocolate manufacturer. This consists of an acid hydrolyzate and an enzyme hydrolyzate of casein and lactalbumin, and thus contains a mixture of amino acids in balanced proportion. An analysis of this preparation is shown in TABLE 1.

The results achieved fully met our expectations. With this product, administered orally, we obtained more rapid results than with milk alone and, even more markedly, than with vegetable protein.

This picture emerges both from studies made by members of our own organization and from the analysis of results obtained by other workers.

The high cost of the protein hydrolyzate is justified and compensated for by the reduction in term of hospitalization that results from its use.

In addition, we have pointed out the urgent need, in certain cases, for rapid treatment and, even though we have not yet obtained enough results to estimate the action of hydrolyzates administered intravenously, we do not hesitate to give infusions of plasma in very serious cases for the immediate purpose of preventing collapse and of re-establishing blood volume.

Dosage

At first we used hydrolyzates in almost pure solution and avoided the use of fats and carbohydrates which, according to some workers, may prolong diarrhea.

Initially, therefore, we administered 50 to 70 gm. of protein in a very unbalanced diet. The results were rapid; the strikingly quick disappearance of edema in 3 or 4 days and of skin lesions in 10 days, coupled with the results of biochemical and histological tests, offered proof of favorable progress.

However, toward the fifth day, a certain number of patients exhibited a particular syndrome, and children who were recovering and beginning to feed themselves suddenly fell into deep coma, with neither convulsions nor neurological symptoms, and died. Serum analysis showed a significant fall in blood glucose level that appears to us to be the cause of the trouble. This can be compared with hypoglycemia found in fatal forms of kwashiorkor, and it indicates the necessity for careful control of carbohydrate metabolism.

On realizing this necessity, from the very beginning of treatment we gave a diet much richer in carbohydrate, one made up of a mixture of partly skimmed milk and protein hydrolyzate. TABLE 2 shows the average results obtained from 20 roughly comparable children.

TABLE 1

ANALYSIS OF PROTEIN HYDROLYZATE PREPARATION

Hydrolyzate

Ingredients	Per cent	
Nitrogen total	12.0	Corresponding to about 88 per cent protein
Amino nitrogen (Van Slyke)	7.0	
Minerals	2.5	Na, 0.9 per cent; Cl, 0.8 per cent
Water	5.0	

100 gm. = 360 cal.

Amino Acid Content

"Essential" amino acids	Per cent	"Nonessential" amino acids	Per cent
1. Tryptophan	0.6	9. Glycine	3.5
2. Phenylalanine	2.5	10. Alanine	6.0
3. Valine	6.0	11. Serine	5.0
4. Leucine	9.0	12. Cystine	0.3
5. Isoleucine	4.7	13. Tyrosine	1.2
6. Threonine	4.0	14. Aspartic acid	4.5
7. Methionine	2.5	15. Glutamic acid	13.0
8. Lysine	7.5	16. Proline	9.0
		17. Histidine	1.6
		18. Arginine	2.6

Even with this carbohydrate-enriched diet, calorie intake at first remains below normal. However, it quickly rises to normal for the age of the child, and is not long in greatly surpassing it, reaching 140 to 150 cal. kg. day.

At this stage, in addition to receiving milk enriched with protein hydrolyzate, the child also follows a normal dietary routine that includes meat or fish, vegetables, fruit, and cheese.

The protein level is high, between 8 and 10, or even 12 gm. kg. day, and is well tolerated, provided the over-all calorie intake is correspondingly increased. It is noteworthy that no digestive troubles develop. On the contrary, the initial diarrhea ceases and the appetite reappears. It seems likely that the introduction of fats into the diet would permit a more rapid increase in calorie intake without causing digestive upsets. A typical case is shown in FIGURE 1.

TABLE 2

STANDARD DIETARY TREATMENT FOR KWASHIORKOR*

	First day	Fourth day	Eighth day	Twelfth day	Sixteenth day	Twentieth day	Twenty-fourth day	Thirtieth day
Proteins (gm.)	35.38	43.15	75.58	88.27	85.96	91.86	86.20	88.60
Carbohydrates (gm.)	35.57	53.21	133.98	147.10	130.91	160.79	138.59	132.47
Fats (gm.)	7.11	10.67	30.35	39.46	45.53	49.49	50.93	54.03
Daily calorie intake	348	478	1,106	1,287	1,278	1,446	1,357	1,381
Cal. from proteins / Total calories (per cent)	41	36	27	27	27	25	25	26
Weight of child (kg.)	10.015		8.476		9.642			11.146

* Twenty typical cases with an average age of 24.46 months.

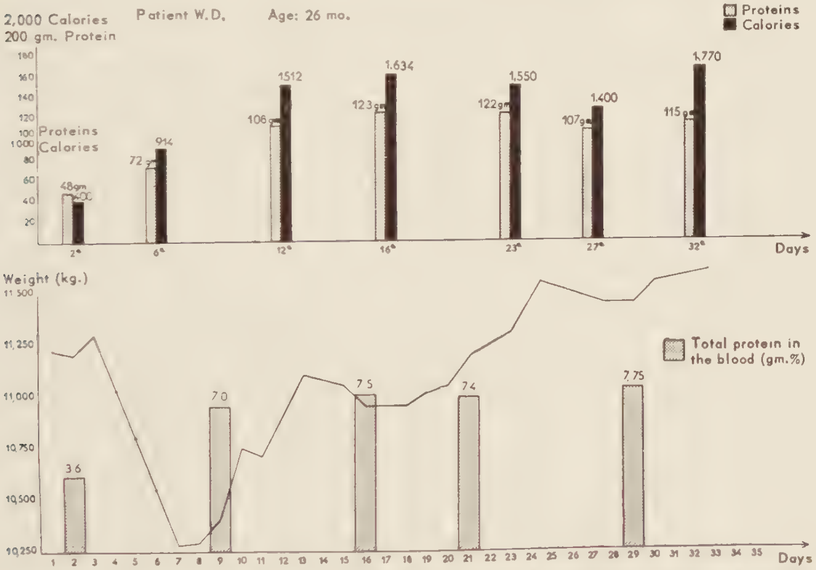

FIGURE 1. Typical case record of a child treated for kwashiorkor by means of a high-protein diet (patient W. D., aged 26 months).

Technique of Administration

When, upon his admission, the condition of the patient gives cause for alarm, we give plasma intravenously an average of 150 ml. The injection is made in the subclavicle vein, using the technique developed by Aubaniac,[9] which seems to us one of the easiest for children of this age.[10]

The child is then put on the diet. It often refuses the bottle and must be forced to take nourishment. A thin polyethylene tube is introduced

TABLE 3

NITROGEN INTAKE AND EXCRETION

Patient		Actual intake					Nitrogen excretion (three days)				
		Calories	Prot. cal./Total cal.	Protein per day	Prot./kg. per day	Nitrogen 3 days	Urine	Feces	Urine/Feces	Balance	Per cent retention
B.M. Age: 22 months Weight:	Days:										
8.45 kg.	1, 2, 3	133.0	41.47	13.8	1.63	6.68	6.27	2.94	2.13	−2.53	
C.G. Age: 22 months Weight:	Days:										
7.80 kg.	2, 3, 4	201.0	42.6	21.4	2.7	10.20	5.60	1.57	3.60	+3.03	29.7
7.80	8, 9, 10	784.0	28.7	56.2	7.2	28.12	11.07	2.97	3.70	+14.08	50.0
8.35	30, 31, 32	493.0	25.7	31.6	3.8	14.68	8.05	1.92	4.20	+4.71	32.0
D. Ous. Age: 20 months Weight:	Days:										
8.00 kg	4, 5, 6	490.5	36.2	44.4	5.5	21.70	10.80	3.60	3.00	+7.30	33.6
8.30	10, 11, 12	843.0	34.0	71.6	8.6	35.05	12.19	9.97	1.20	+12.89	36.6
9.50	39, 40, 41	990.0	25.9	57.6	6.0	27.45	17.30	5.10	3.40	+5.05	18.4
B.N. Age: 20 months Weight:	Days:										
9.50 kg.	2, 3, 4	298.0	42.4	31.6	3.3	15.11	6.80	2.30	2.95	+6.01	39.7
10.00	15, 16, 17	823.0	32.4	66.7	6.6	32.70	18.00	5.90	3.00	+8.80	26.9
11.00	29, 30, 31	995.0	25.0	62.3	5.6	29.49	17.30	2.40	7.20	+9.79	33.1
11.25	44, 45, 46	754.0	24.9	46.9	4.1	22.15	12.10	2.73	4.40	+7.32	33.0
A.N'D. Age: 19 months Weight:	Days:										
10.75	1, 2, 3	330.0	42.4	35.0	3.25	16.61	4.50	3.00	1.50	+9.11	54.8
11.25	16, 17, 18	1152.0	25.8	74.5	6.62	36.63	9.20	3.70	2.50	+23.70	64.7
12.25	30, 31, 32	1082.0	23.02	62.3	5.08	29.49	8.66	4.40	1.96	+16.43	55.7
D. Oum. Age: 25 months Weight:	Days:										
7.75	1, 2, 3	122.5	51.6	15.8	2.03	7.68	4.50	1.50	3.00	+1.68	21.8
8.65	35, 36, 37	1182.5	23.8	70.5	8.15	33.46	12.30	5.50	2.20	+15.66	46.8
9.35	49, 50, 51	1275.0	23.8	75.8	8.10	35.77	12.40	5.60	2.20	+17.77	49.6

through the nose, and a mixture of skimmed milk and hydrolyzate is gradually administered. The tube can be left in place for a day or two, and this procedure is less injurious than if the tube is reinserted every three hours. We must not forget that we are dealing with weakened children, and that nursing care is made easier for a small staff if the tube is left in place.

After 24 to 48 hours the child begins to eat voluntarily; forced feeding is then no longer necessary. Feeding is carried on by the bottle, and then meals are added, thus permitting an increase in total intake. After the twelfth day the child himself can regulate his intake, which thus becomes more irregular, although still in excess of the average for the child's age group.

Discussion of the Level of Protein Diet

These high levels of protein intake contradict the normally accepted ideas of pediatricians concerning the level of protein tolerance.

The question arises, however, as to whether or not these dosages are necessary. Is it possible to obtain identical results with smaller dosage? In addition, is there not a danger in prescribing a diet so rich in proteins?

In the course of treatment, the blood urea level rises continuously and increases from 10 or 15 mg. to 35 to 40 mg. per cent. It has been known to increase even beyond these levels and, in a few cases, has reached 80 and even 100 mg. Dean[11, 12] observed the same phenomenon and, without modifying the diet, found that the level returned to normal after a few days. In view of these high levels, we considered it necessary to reduce protein intake and, after some days, the urea level subsided. This increase in blood urea level is not accompanied by any deleterious side effects and appears to be the result of a nitrogen metabolism operating at full force.

At the conference on nutrition held in Princeton, N. J., in 1955,[13] Platt pointed out the danger of suddenly administering a high-protein diet to animals formerly deprived of sufficient protein, since this might bring about renal lesions.

Measurement of nitrogen intake and excretion has shed light on certain points. The results obtained are set forth in TABLE 3. They already have been published,[14–16] and I shall touch only the principal points.

The nitrogen balance was always positive, except in the case of one child who proved impossible to feed and therefore died. The ratio of urinary nitrogen to fecal nitrogen is greater than one. We can conclude from this that intestinal absorption is good. From this we deduce that these quick results are obtained by using protein hydrolyzates that do not at first necessitate intensive action in the atrophied exocrine cells of the pancreas.

Nitrogen balance remains strongly positive for several weeks, and nitrogen retention reaches 50 to 60 per cent by the fiftieth day (patient D. Oum.). It would seem, therefore, that a high-protein diet is beneficial.

The best levels of nitrogen retention are obtained when the components of the diet—carbohydrates, fats, and proteins reach a certain balance. A heavy relative intake of protein is tolerated only provided total caloric intake is increased.

There seems to be a link between percentage retention of nitrogen and the ratio of protein calories to total calories and, particularly in the No. 2 balances (reached from the eighth to the eighteenth day), the percentage of retention diminishes proportionately with the increase in ratio.

Nitrogen retention does not, therefore, depend solely on the quantity of protein ingested, but is also influenced by the ratio of protein calories to total calories, and it seems to be optimal when this ratio reaches 25 per cent.

Measurement of the tyrosine index and of creatinine and creatine in the urine showed that there were no biological symptoms of intolerance to nitrogen.

Consequently, treatment by means of a high-protein diet does not appear to overstrain liver function, and it would seem useful to prolong the diet since nitrogen retention remains high. This procedure does not seem to be dangerous.

Supplementary Treatment

The addition of vitamins, particularly vitamin B_{12}, to a high-protein diet has been recommended. However, treatment by means of isolated vitamins or groups of vitamins in no way alleviates the condition. In particular, the addition of vitamin B_1 and B_2 does not affect cheilitis, whereas a high-protein diet causes it to disappear within a few days. The same is true of niacin, which has no effect on the skin disorders.

The addition of vitamins does not appear to enhance the action of a high-protein diet which, with the addition of hydrolyzates, is particularly rapid. The same is true of hormones, particularly androgens, which are recommended because of their anabolic action on proteins.

Antibiotics are frequently prescribed to control infection that has often characterized the onset of kwashiorkor or that is associated with it. On the other hand, antiparasitic agents are not used until after several days of treatment, when the general condition of the child is improved except in cases where serious parasitic diseases, such as certain forms of amebiasis and malaria, are jeopardizing the child's life. More recently, potassium has been added to increase the over-all benefit of this treatment.

Analyses of blood-potassium levels in patients at the time of their admission often give low figures - less than 2 mEq. Average figures obtained from 25 patients are shown in TABLE 4.

In similar fashion, analyses of urine conducted in connection with nitrogen-balance tests have allowed us to evaluate the elimination of electrolytes at the onset of kwashiorkor and at the time of the re-establishment of normal nitrogen level (TABLE 5).

At admission, there exists a feeble hypotonia in the extracellular fluid but, as soon as the edema disappears, tonicity becomes normal once again. However, sodium level remains slightly below normal, and the elimination of sodium in the urine is not great. There is even a decided preponderance of chloride ion over sodium ion. The serum potassium level is low at first and increases as the result of the addition of potassium during treatment. In certain cases, however, potassium does not seem to be retained, and the serum potassium level increases hardly at all, whereas the potassium level in the urine is high. It seem to us that the addition of other cellular ions such as phosphorus, sulfur, and magnesium should facilitate the retention of potassium.

Table 4
Electrolyte Composition of Serum of 25 Kwashiorkor Patients

Period of treatment	Freezing point (C.) depression (corrected for urea)	Parts per thousand		
		Cl	Na	K
Average normal limit	−0.560°	98 to 107	135 to 155	4.4 to 5.2
Average level	−0.560°	104	145	4.8
First period* (admission)				
Minimum	−0.490°	87	119	2.6
Maximum	−0.555°	105	140	5.6
Average	−0.530°	99	131	3.8
Second period† (5th to 12th day)				
Minimum	−0.535°	95	120	4.4
Maximum	−0.560°	105	139	6.4
Average	−0.545°	100	128	5.1
Third period (after 20 days)				
Minimum	−0.540°	92	133	4.5
Maximum	−0.565°	104	144	5.4
Average	−0.550°	100	136	4.8

* Corresponds with the day of admission and with days 1, 2, and 3 of treatment.
† Corresponds to the time of minimum weight, or about the eighth day.

Table 5
Urine Analysis—Samples Taken for Quantitative Nitrogen Balance

Period	Volume, 24 hours (mean of 3 24-hr. periods)	Level, mEq./liter			Amount eliminated in 24 hrs., mEq.				
		Cl	Na	K	Cl	Na	K	Na/Cl	Na/K
First period*									
Minimum	0.070	10.9	1.4	0.08					
Maximum	0.540	49.0	7.9	57.9					
Average	0.345	27.0	4.9	27.4	9.3	1.7	9.45	0.18	0.17
Second period* (at minimum weight)									
Minimum	0.200	21.60	2.2	2.7					
Maximum	0.650	42.25	39.5	153.8					
Average	0.415	20.30	14.1	45.1	8.4	5.8	18.70	0.69	0.31
Third period (after 1 month)									
Minimum	0.200	22.80	11.6	1.5					
Maximum	0.385	51.26	20.3	11.5					
Average	0.297	32.10	19.9	8.4	9.5	5.9	2.50	0.62	2.37

* In the first and second periods, the increase in urine flow results from the cessation of edema.

The fall in potassium level is therefore the most striking characteristic of electrolyte disorder, and perhaps the sodium is retained to replace it in the cells. The K deficiency is caused by diarrhea and vomiting, and is aggravated by anorexia. It is suggested also that corticoadrenal hormones, particularly

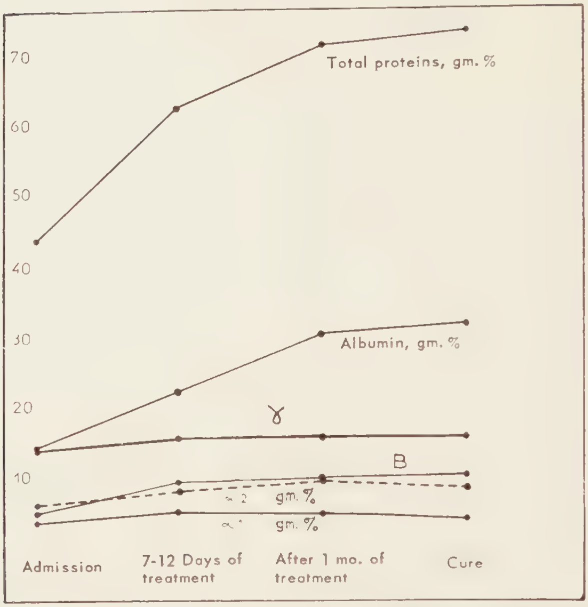

FIGURE 2. Serum protein changes during the treatment of kwashiorkor (grams per thousand).

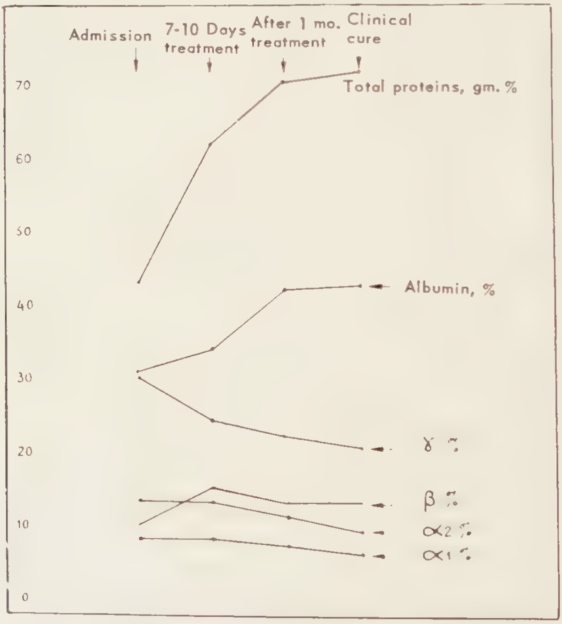

FIGURE 3. Serum protein changes during the treatment of kwashiorkor (grams per hundred).

aldosterone, accentuate the elimination of potassium while causing sodium to be retained. As soon as tissue repair begins, the need for potassium increases.

It is therefore necessary to add significant doses of potassium (100 to 200 mg. kg. day), preferably in citrate or acetate form, to correct the acid-producing tendency of the high-protein diet (the normal alkaline reserve tends to decline slightly at the beginning).

Results

The effectiveness of this treatment is supported by the following clinical evidence:

(1) The disappearance of edema is less rapid than when pure hydrolyzate solutions are used, and the weight curve reaches its lowest point in 5 to 8 days. In addition, in cases of slight edema, the curve, instead of dipping, stays level before making a decided turn upward, and the gain in weight compensates for the loss sustained through the disappearance of edema.

(2) The appetite recovers.

(3) There is cessation of diarrhea and a change from fermentative to proteolytic intestinal flora.

(4) A decrease in psychological disorders occurs. For example, a child, once uncooperative and apathetic, begins to smile and to play.

(5) There is disappearance of disorders of the skin and of mucous membranes; in 10 days the skin ceases to peel. Skin lesions are healed, and only areas of depigmentation in process of healing persist. Treatment must be carried on for an entire month, however, before the skin becomes supple, shiny, and pigmented and takes on a completely normal appearance.

Biological symptoms develop simultaneously with clinical symptoms. The serum-protein level rises from 4 to 7 gm. per cent in a week, as shown in FIGURES 2, 3, and 4 and in TABLE 6.

Above all, it is the increase in the albumin that is striking. On the electrophoretic curve, in addition to the albumin peak, a fall in the γ_1- and γ_2-globulins and a rise in the β-globulins are observed. These indications appear to be sufficiently uniform to provide a reliable means of following the progress of the disease. (FIGURE 5).

The enzyme levels also rise, especially in the case of the blood amylase level, which is low at the beginning of treatment and rises rapidly, passing the normal level of 80 to 100 units to reach an eventual level of 150 to 200 units.

Finally, the carrying out of liver biopsy affords us the means of observing the regression of steatosis. The change here is extremely rapid. In 8 days, general steatosis of the lobule disappears, leaving the liver normal and the protoplasm of the cells barely clarified.

Under present conditions, even with a shortage of well-trained staff, treatment with a high-protein diet has reduced the death rate considerably (from 80 to 50 per cent). Under the strict control of a nurse trained in dietetics, the administration of a high-protein diet has resulted in a fall in the death rate to 25 per cent.

During the past few months, judicious use of antibiotics and of potassium has reduced the death rate to 20 per cent. This residual mortality rate is due either to the admission to the hospital of patients who die within a few hours or to infection, the role of which in aggravating malnutrition we have already noted. These infections, following their own course, may cause death in spite of the use of antibiotics.

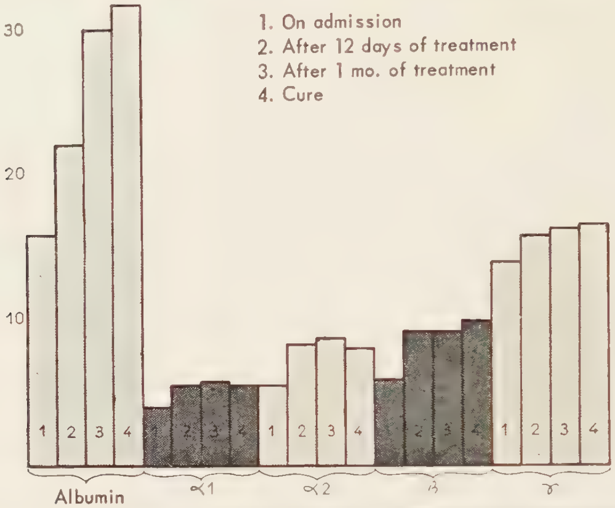

FIGURE 4. Variation in serum protein level during treatment of kwashiorkor (grams per thousand). Symbols: 1, on admission; 2, after 12 days of treatment; 3, after 1 month of treatment; 4, cure.

TABLE 6
THE RISE OF SERUM PROTEIN WITH TREATMENT

	Number of analyses	Average	Minimum and maximum figures	
At admission	89	4.28	2.6	6.2
After 5 to 12 days of treatment	64	6.02	3.2	7.7
	6 (1953)*	5.05		
	24 (1954)*	6.14		
	20 (1955)*	7.08		
After 1 month of treatment	53	7.04	8.6	6.1

* We have purposely indicated the figures obtained in 1953, 1954, and 1955 after 1 week of treatment. According to my interpretation, these data demonstrate the progress we have made in our method of treatment.

However, the prognosis for these children remains uncertain. It would nevertheless seem that there is no direct relationship between fatty degeneration of the liver and fibrosis, which develops so often in somewhat older children.

Thus, the immediate rather than the distant future of these children should cause us concern; relapse is particularly to be avoided. On returning home, the child is exposed once again to the poor habits and ignorance of its parents, and it is then that preventive treatment becomes important.

PREVENTIVE TREATMENT

The prevention of kwashiorkor therefore appears to be of greater moment than does the treatment of advanced forms of the disease.

Preventive treatment is even more important in the case of the ill-defined forms that occur so frequently. In a recent survey, we found, with L. Aubry,[17] that, in the weeks following weaning, one child in every four shows signs of malnutrition: edema, disorders connected with the skin and the mucous membrane, and deterioration of the hair, all of which rarely form a complete picture, but are sufficiently marked to indicate a certain degree of deficiency.

Moreover, it has been our experience in several cases in which the prescribed diet has not been followed that severe malnutrition can ensue, thus requiring immediate hospitalization of the patient. The study of the weight curve of the African child shows that dietary deficiencies start very early in life.

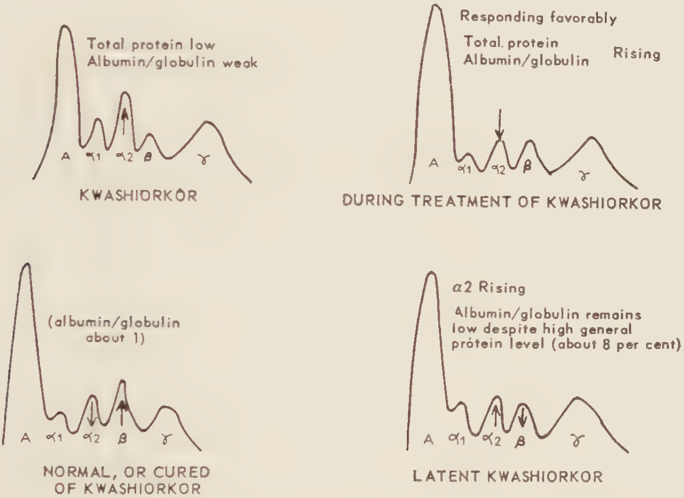

FIGURE 5. Variations of the electrophoretic curve in kwashiorkor.

During the first three months of life, the breast-fed child, in close contact with the mother, develops well. Its weight curve is better than that of the European child, as shown in graphs shown by Falkner at the Copenhagen Congress of Pediatrics (1956).[18, 19] At 3 months, the 2 curves meet and, from then on, the curve of the African is consistently lower. This retardation, barely indicated at 6 months, becomes more pronounced up to 1 year or 18 months. The cause is obvious: the mother's milk has become insufficient, and the African mother does not know how to supplement her child's diet. Her milk remains of good quality, and research, undertaken particularly in the Belgian Congo, has established that its nitrogen and amino acid content remains unchanged in spite of the continuation of breast feeding. However, the quantity of the milk diminishes, and some workers have suggested that the mother should be given supplementary nutrients to increase her production of milk.

However, in trials conducted by Holemans, Lambrechts and Martin,[20] it was found that for every 40 gm. of skim milk received daily by the mother, the child receives only 0.8 gm. of supplementary proteins.

In addition, even if the quantity of milk were to be increased, it remains true that this excellent food is by itself insufficient to assure normal growth after the age of 6 months.

It would appear, therefore, that the efforts of the PMI* should tend rather toward supplementing the child's food than adding to the diet of the mother.

Moreover, at weaning, a point that must be reached sooner or later, the problem becomes even more acute. This is the most dangerous time of all for the child, since he is then deprived of his mother's milk without having learned to replace it by an adult-type diet that has been introduced progressively. The child then lives for several months in a state of balance or of slight deficiency. After several months he may adapt himself to the situation, and slow or limited growth may resume. On the other hand, if a parasitical or infectious condition gains a hold at this time, the balance is immediately disrupted and kwashiorkor makes its appearance. In 1956 more than one half of our kwashiorkor patients were children between the ages of 18 and 30 months who had just had measles.

The preventive treatment of kwashiorkor must take two considerations into account: the education of the people, especially of the mothers, in good feeding habits, and supplementation of the local basic diet with a product that will supply the missing amino acids.

Education in Good Feeding Habits

I do not propose to devote much space to this subject in this report. A whole program of education has been set up in Dakar that is concerned primarily with the training of personnel, without whom no program can be put into effect. Various tests conducted by the Food and Agriculture Organization (FAO) of the United Nations in French West Africa have made possible the formulation of a procedure that can be applied at all levels where PMI has any influence: in the hospital, in the clinic, in PMI centers, in the home, and in the school. The use of photographs and of locally drawn posters helps in the education of the population. Radio broadcasts in the native language are also used.

Introduction of Local Products into the Diet

Supplementing the local basic diet with a product that will supply the missing amino acids can be achieved either empirically by introducing local products into the diet—this procedure I shall consider first—or by the addition of amino acids both singly and in groups. This latter procedure does not lend itself easily to scientific evaluation, and thus far we are able to present only inconclusive results.

The problem of providing a sufficiently high protein intake for the entire population rightly claims the attention of government. The authorities of every area are seeking the most readily attainable solution of this problem.

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* Protection Maternelle et Infantile (Mother and Child Health Service).

Milk supplementation is hardly possible as a long-term solution. A local milk industry would require years before it could become established, and it would be hampered by poor pasture land, animal disease, and difficulties of transportation and harvesting.

Dry milk has proved its effectiveness in trials carried out by the United Nations International Children's Emergency Fund (UNICEF). It is, however, too expensive to be used widely by the native population except when it is provided free of charge by such an international organization. Consequently, most countries are trying to develop the raising of livestock, the formation of a fishing industry, or the cultivation of selected cereals or vegetables.

In French West Africa, millet or sorghum form the basic diet, with corn as a secondary foodstuff. However, the use of locally grown or imported rice is beginning to spread.

Apart from these basic cereals, two protein sources exist that have not thus far been exploited. The first of these is peanut press cake, a by-product of oil production, which is available in large quantities. Peanut production in French West Africa in 1948 was 870,000 metric tons, and in 1956 and 1957, Senegal alone produced 600,000 metric tons. Peanut press cake is rich in protein (it has an average protein content of 50 per cent), and until now it has been exported to France or to other foreign countries for use as cattle food. The press cake that remains after extraction with chemical solvents can be dangerous; residual trace quantities of solvent render it carcinogenic. We must therefore rely on press cake from which the oil has been extracted by means of pressure and under conditions in which the heat used has been carefully controlled to avoid the destruction of lysine.

Simple processing of the press cake (removal of the husk, roasting, and sieving) gives a product suitable for human consumption containing less than 2 per cent cellulose and 5 per cent fat. A commission of the FAO has established production standards, and the tests that we have conducted in French West Africa over the past four years have been made with this standard product.

The second most important dietary protein supplement is fish. A considerable fishing industry already exists in coastal areas. The African dries the fish and eats it when the season is no longer suitable for fishing. Small quantities of this dried fish are exported into the interior, but this food product is preserved and transported under very poor conditions.

The development of this source of protein is very attractive, since it can supplement the diet more effectively with the essential amino acids than can vegetable protein. Fish is most conveniently used in the form of fish flour, which is already in industrial production in several areas of Africa, including South Africa, and Morocco. UNICEF has recently advanced funds for the construction of a pilot plant in Chile scheduled to begin production in September 1957.

For the past four years we have used fish flour produced on a small scale by the Fisheries Service of the Government General of French West Africa.

In trials conducted with children we have been able to ascertain which varieties prove the most acceptable and what the tolerance level is. The latter is greatly in excess of effective dosage.

For the past three years, we have used fish flour mixed with millet and peanuts. These two products add greatly to the protein content of the diet—50 per cent in the case of peanuts and 70 to 80 per cent in the case of fish flour. However, these proteins are not of equal value.

TABLE 7 presents the amino acid composition of millet (the basic food in our region of Africa), of peanut press cake, and of fish (possible supplementary foodstuffs) and, for purposes of comparison, eggs (recommended by Mitchell and Block) and milk (recommended by the Conference on Nutrition held in Princeton, N.J., in 1955[13]).

As the protein content varies according to the product, it is helpful in calculations to have the use of the listing shown in TABLE 8, which gives values for the amino acids in 100 gm. of product.

TABLE 7
COMPARISON OF AMINO ACID CONTENT
(Calculated on 100 gm. protein)

	Peanut[21]	Millet[22]	Fish[23]	Cow's milk[24]	Egg[25]
Arginine	9.95	4.70	8.27	3.5	6.4
Cystine	1.6	1.25		0.7	2.4
Histidine	2.1	2.10	2.63	2.4	2.1
Isoleucine	3.0	4.30	6.00	6.6	8.0
Leucine	7.0	17.40	9.27	11.8	9.2
Lysine	3.0	2.60	9.72	8.1	7.2
Methionine	1.15	2.60	2.63	2.2	4.1
Phenylalanine	5.2	4.90	4.45	4.6	6.3
Threonine	1.95	4.90	5.18	4.8	4.9
Tryptophan	0.95	2.30	0.81	1.4	1.5
Tyrosine	4.4	2.00	2.54	6.2	4.5
Valine	8.0	5.65	6.54		7.3

In comparing the amino acid content of a product with that of egg, it is possible to express the difference percentagewise and, in particular, to determine the percentage deficiency of a limiting factor. Thus, the protein value is inversely proportional to the percentage deficiency of the limiting factor.

The percentage of amino acids found in millet and peanuts compared with those found in egg protein is shown in TABLE 9. Lysine forms the chief deficiency in millet, and cystine and isoleucine are secondary limiting factors. On the other hand, millet is rich in leucine and tryptophan.

Methionine is the chief limiting factor in peanuts, but four other amino acids are deficient to more than 50 per cent. In order, they are: isoleucine, lysine, threonine, and cystine.

The purpose of supplementation is to lessen deficiency. In mixtures containing two proteins of differing amino acid composition, each tends to compensate for the deficiency of the other, and the whole becomes of greater value.

Millet supplements methionine deficiency in peanuts, and peanuts contain a little more lysine than does millet. Theoretically, however, peanuts do not appear to be a good supplement for millet.

However, we must not forget that peanut press cake contains 50 per cent protein and, in addition, some B vitamins. On the other hand, the phosphorus:calcium ratio is not good, as shown in TABLE 10.

TABLE 8
COMPARISON OF AMINO ACID CONTENT
(Calculated for 100 gm. of product)

	Peanuts, 50% protein	Millet, 10% protein	Fish, 70% protein	Cow's milk, 3.5% protein	Eggs, 13% protein
Arginine	4.97	0.470	5.79	0.122	0.832
Cystine	0.80	0.125		0.024	0.312
Histidine	1.05	0.210	1.84	0.084	0.273
Isoleucine	1.50	0.43	4.20	0.227	1.04
Leucine	3.50	1.74	6.49	0.413	1.19
Lysine	1.50	0.26	6.80	0.283	0.936
Methionine	0.575	0.26	1.84	0.077	0.533
Phenylalanine	2.60	0.49	3.11	0.161	0.819
Threonine	0.975	0.49	3.62	0.168	0.637
Tryptophan	0.475	0.23	0.567	0.049	0.195
Tyrosine	2.20	0.20	1.78	0.217	0.585
Valine	4.0	0.565	4.58		0.949

TABLE 9
THE PERCENTAGE OF AMINO ACIDS FOUND IN MILLET AND IN PEANUTS COMPARED TO THOSE FOUND IN EGG PROTEIN[28]

	Millet (*Pennisetum*)	Peanuts
Arginine	−26	
Cystine	−48	−53
Histidine	0	
Isoleucine	−46	−63
Leucine	+90	
Lysine	−64	−58
Methionine	−36	−73
Phenylalanine	−22	
Threonine	0	−58
Tryptophan	+54	
Valine	−22	

It is of benefit, however, to combine fish with millet or with peanuts. This produces a protein-rich food with well-balanced amino acids, and the deficiency of the most limiting factor, although varying with the kind of fish, does not exceed 50 per cent. In addition, the limiting factors are different from those of cereals. Thus the lysine content is very high greater, in fact, than that in eggs. On the other hand, the tryptophan level is low, but fortunately this is compensated for by the millet. The level of methionine content, although superior to that in milk, is considerably less than that in eggs.

It is on the basis of these facts that we have undertaken work with the African child. The first results of this work were announced at the nutrition conference held in Luanda, Angola, in 1956.[29]

In the study of the supplementation of the diet of infants, three factors must be defined: (1) the acceptability and the immediate tolerance of the digestive system, (2) tolerance in the face of prolonged administration, and (3) the effectiveness of the product in supplementing the conventional diet.

Although these tests can be conducted with healthy children in schools, camps, orphanages, and nurseries and the effect of the diet can be measured by growth, and although the tests are most valuable in subjects of this age, we unfortunately have no nursery for small children, which makes testing impossible. In the hospital service it is possible to enforce strict control, but only at the cost of increasing the length of stay of the children who are recovering or of hospitalizing healthy children, which is not recommended.

TABLE 10

VITAMINS IN MG. PER 100 GM.

Vitamins	Thiamine	Riboflavin	Niacin
Peanut press cake[21]	0.84	0.12	16
Millet[26]	0.33	0.15	2.1

MINERALS

	Ca	P	Ca/P	Fe
Peanut press cake (per 100 gm.)[27]	0.13	0.8	0.16	0.0035
Millet (per 100 gm. ash)[28]	1.00	21.8	0.05	

We have thus preferred to conduct tests with children receiving a deficient diet, on the theory that a product capable of rectifying deficiencies can prevent them, and also because it is easier and quicker to estimate the effectiveness of treatment by measuring the rate of disappearance of pathologic symptoms than by measuring the rate of growth of the child.

We have therefore given children with incipient kwashiorkor a supplementary diet, and we have carefully followed their weight curves, observed any clinical symptoms, measured protein level in the blood, and have sometimes observed fatty degeneration of the liver by biopsy. These children were often suffering from an infectious or parasitic disease that required treatment. Weight curves were distorted by the disappearance of edema, and many observations proved useless, since the children did not follow the diet regularly.

Nevertheless, 4 kinds of supplementation were studied simultaneously in 4 groups of children at PMI clinics. The first group was given millet alone. This was a control group in which the children were given supplementary calories, but no additional proteins. The fourth group was also a control and received the same supplementary calories, but with the addition in this case of 16.5 gm. of milk protein. Group 2 received millet plus peanut press cake, and Group 3 received millet plus peanut press cake and fish.

TABLE 11

TRIAL SUPPLEMENTED DIETS

	Grams	Protein	Calories
Group *1: millet alone*			
Flour	60	4.72	198.2
Sugar	5	—	20
Total	65	4.72	208.2
Group *2: millet plus peanut press cake*			
Millet and peanut press cake	49.2	8.83	184.5
Sugar (18 per cent)	10.8		43.2
Total	60.0	8.83	227.7
Group *3: millet plus peanut press cake plus fish flour*			
Millet and peanut press cake	45.76	8.21	171.61
Fish (7 per cent)	4.2	3.65	15.33
Sugar	10.04		40.16
Total	60	11.86	227.10
Group *4: skim milk*			
Milk powder	46	16.56	165.6
Sugar	10		40
Total	56	16.56	205.6

TABLE 12

COMPOSITION OF DIFFERENT DIETS USED AT THE PMI CENTER

(Daily ration in grams)

	Group 1 Millet Rouge	Group 2 Millet + peanut press cake[2]	Group 3 Millet + peanut press cake + fish flour[23]	Group 4 Skim milk[3]	Theoretical needs per 10 kg.[31]
Protein	4.72	8.83	11.86	16.56	
Arginine	0.22	0.71	0.93	0.68	
Cystine	0.06	0.13	0.19	0.16	1.26
Histidine	0.10	0.19	0.27	0.42	
Isoleucine	0.20	0.53	0.70	1.21	0.63
Leucine	0.82	0.64	0.94	1.79	0.90*
Lysine	0.12	0.25	0.59	1.41	4.25
Methionine	0.12	0.16	0.24	0.51	1.70
Phenylalanine	0.23	0.45	0.58	0.89	0.85
Threonine	0.23	0.28	0.45	0.76	1.69
Tryptophan	0.11	0.14	0.16	0.24	0.87
Tyrosine		0.22	0.30	0.97	0.30
Valine	0.27	0.62	0.82	1.13	1.61
Glycine		0.29	0.266		

* Leucine altered in 1956 to figure suggested by Autret.[35]

The composition of the different diets tried is shown in TABLES 11 and 12, and the first results are shown in TABLE 13.

Since the experiment was not completed, a statistical estimate could not be made. It would seem, however, that millet-peanut and millet-peanut-fish mixtures are definitely superior to millet alone, which is in accordance with theory. We found, however, that good results were obtained in 10 cases fed on millet alone.

The results obtained with skim milk were rather surprising, and the gain in weight was less than that obtained with the mixtures.

The quality of the milk is not critical, and the only other factor that differentiated this group from the others was that of age (the children in Group 4 were younger than those in the 3 other groups).

Table 13

Comparative Action of Different Diets

	Number	Average age	Average weight	Average duration (in days)	Increase in weight (gm.)	
					Total	Per day
Millet alone						
Failures	16	23.2	8.294	15.6	0	0
Good (without edema)	3	32.5	9.000	14	533	30.9
Good (with edema)	7	20.6	9.005	25.4	373	14.6
Total and average	26	22	8.539	18.1	(−575.5/4)* 150	8.30
Millet and peanut press cake						
Failures	29	21.3	7.543	20.5	0	0
Good (without edema)	46	21.5	7.770	34.1	860	25.18
Good (with edema)	67	21.2	8.396	26.9	552	20.50
Total and average	142	21.3	8.019	27.9	(−710/33)* 539	19.28
Millet and peanut press cake and fish flour						
Failures	10	21.8	8.128	21.0	0	0
Good (without edema)	8	20.5	7.696	28.2	1120	39.7
Good (with edema)	33	22.6	8.996	25.7	688	21.07
Total and average	51	22.1	8.618	25.2	(−494/17)* 527	20.9
Skim milk						
Failures	5	15.8	7.216	21.4	0	0
Good (without edema)	6	18.8	8.250	18.3	366	20
Good (with edema)	10	22.7	9.507	29.9	515	17.2
Total and average	21	19.9	8.602	24.6	(−451/7)* 350	14.24

* These negative quantities indicate initial losses in weight in grams per individual for the number of children indicated. Thus, 4 children each suffered an average temporary loss of 575.5 gm. due to reduction of edema at the beginning of treatment.

The rather surprising results obtained with this group also may be attributable, in part, to the small number of cases that we were able to follow after their discharge. This, as I pointed out earlier, makes interpretation difficult.

In addition to these tests, we should take note of trials conducted with prepared mixtures consisting of cereals, vegetables, skim milk, and fish flour. At the moment, we are testing the effectiveness of "cookies" made of millet, peanut press cake and fish flour, which are easy to distribute among the school age population.

In addition to this rather empirical method, we have also tried to determine which amino acids are deficient so that, in other tests, we could add to the basic diet a single amino acid or an amino acid mixture rather than a single protein or protein mixture.

We attacked this problem in 4 different ways: (1) by nutritional inquiries, (2) by measuring and analyzing the therapeutic diet for kwashiorkor patients, (3) by chromatography of the serum and urine of kwashiorkor patients, and (4) by supplementing the basic diet with amino acids.

Nutritional Inquiries

Numerous nutritional inquiries have been undertaken by ORANA.* These studies were done principally on family groups (3 groups were established: the first consisting of from 2 to 5.99 persons, the second, 6 to 8.99 persons, and the third more than 9 persons). The studies showed the effect on diet of social position, of seasonal changes, of the number of children, of religion, of the activity of the head of the family, and of the family's resources.

It will be seen from this list of factors how difficult it is to determine the average family intake. In collaboration with ORANA, we have carried out some researches on individuals in order to determine the exact intake of a child suffering from latent kwashiorkor, but in these cases new difficulties are added to those encountered in research on a family-group level. The child feeds from the communal dish; in order to measure what he receives, this custom must be changed so that the child can eat by himself, which will obviously modify his intake. Under such conditions it is possible to weigh only cooked food. Trials have shown that there is considerable variation from day to day, and it is therefore necessary to have a trained person with the child day and night for at least 8 days. This cannot be accomplished without seriously disrupting family life.

On making a detailed account of the products eaten, it becomes apparent that the diet of many children has never before been submitted to detailed analysis, and that its amino acid, mineral, and vitamin composition remains unknown. Data that do exist on these points vary with the year, the region, the techniques used, and the worker, and most of the data are given for the products of the diet in the raw state.

In order to illustrate the variation found in analyses of these products, we reproduce, in TABLE 14, the various results obtained in the analysis of peanut press cake.

All of these accumulated difficulties mean that it is scarcely possible to derive any definite information from these long and difficult studies. We know that the diet is unbalanced, rich in carbohydrates, and poor in proteins. It is difficult to determine whether or not the calorie intake is sufficiently high, and even more difficult to determine which amino acids are deficient and to what degree.

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* Office de Recherche de l'Alimentation et de la Nutrition Africaines, Dakar, French West Africa.

Therapeutic Diet in the Treatment of Kwashiorkor

It is easy to calculate the amino acid content of the standard therapeutic diet that brings about a rapid cure of kwashiorkor. TABLE 15 gives figures for the sixth day of a diet of skim milk plus hydrolyzate. These figures are much in excess of known requirements. It will be noted that lysine and isoleucine are present in great excess over theoretical requirements.

TABLE 15 should allow us to regulate the diet and bring it more into accord with theoretical requirements. The existing proportions of different amino acids should be respected, and we hope to treat kwashiorkor by the addition of amino acids in these proportions.

TABLE 14

AMINO ACIDS IN PEANUTS*

Amino Acids	O.K.Kaden[37]	Borden's Review[38]	Ferrando & Jacquot[27] (p. 550)	Baudouin[39]	Busson[40]
Arginine	+	11.3	9.95	10.4	4.36
Cystine	+	1.4	1.6	1.9	?
Histidine	?	2.2	2.1	5.7	1.07
Isoleucine	+	4.1	3.0	?	2.07
Leucine	?	7.1	7.0	?	3.81
Lysine	+	3.5	3.0	6.0	1.74
Methionine	+	0.8	1.15	0.5	0.50
Phenylalanine	+	4.9	5.2	4.6	2.39
Threonine	+	2.8	1.95	1.6	1.31
Tryptophan	?	0.8	0.95	1.8	?
Tyrosine	+	2.9	4.4	?	1.92
Valine	+	4.6	8.0	3.7	2.39
Proline	+	5.0	?		3.55
Serine	+	?	?		2.80
Glutamic acid	+	18.3	?		8.17
Aspartic acid	?	13.0	?		5.39
Glycine	+	?	5.6	2.6	?

* Percentages based on 16 gm. of nitrogen.

Chromatography of Blood and Urine

By means of serum chromatography, we have tried to determine the variations in amino acid levels that develop during kwashiorkor. We used paper chromatography and followed the technique developed by Lissitzky, Césaire, and Massonet.[32, 33]

A correlation was always found between the intensity and the number of spots, on the one hand, and the level of amino acids in the blood, on the other. The disadvantages of this technique are well known, and it is not possible to distinguish all the amino acids present. Tryptophan is destroyed by the initial acid hydrolysis, methionine is partially oxidized, and it is difficult to distinguish the sulfur amino acids.

Taking these disadvantages into account and comparing results obtained in the treatment of kwashiorkor patients (TABLE 16), with those obtained in controls or given in the literature, it would seem that a certain number of amino acids are present in blood serum, including leucine, valine, alanine, proline, threonine, glycine, serine, and glutamic acid whereas, on the other hand, lysine and arginine are often not present. Unfortunately, this technique reveals no information on those amino acids that are not normally distinguishable, such as histidine, isoleucine, phenylalanine, tryptophan, and methionine, and yet three of these are essential amino acids.

TABLE 15

AMINO ACIDS FURNISHED BY THERAPEUTIC DIET*

Amino acids	156 gm. of partly skimmed milk powder (20 per cent protein content)	Protein hydrolyzate (36 gm.)	Total	Theoretical needs of 9-kg. child (according to Albanese)†
Arginine	1.092	0.936	2.028	1.134
Cystine	0.218	0.108	0.326	—
Histidine	0.748	0.576	1.324	0.567
Isoleucine	2.028	1.692	3.720	0.810
Leucine	3.681	3.240	6.921	3.825
Methionine	0.686	0.90	1.586	0.765
Phenylalanine	1.435	0.90	2.335	1.521
Threonine	1.497	1.440	2.937	0.783
Tryptophan	0.436	0.216	0.652	0.270
Valine	1.934	2.160	4.094	1.449
Lysine	2.527	2.70	5.227	1.530

* Skim milk plus hydrolyzate (sixth day, in grams).

† Four considerations must be kept in mind with regard to these figures of Albanese.[31] (1) In reaching his figures, he assumed a diet higher in calories than is necessary when natural proteins are used; (2) the requirements for amino acids vary from child to child; (3) the results of his short-term experiment cannot be projected over a long period of time; and (4) his data apply to normal children, and thus they cannot be used for children suffering from nutritional deficiencies.

TABLE 16

CHROMATOGRAPHY OF SERUM AMINO ACIDS

Amino acid	Accepted data	Dakar controls	Kwashiorkor	Presence*
Leucine	+	7/7	17/17	P
Valine	+	7/7	17/17	P
Alanine	+	7/7	17/17	P
Proline	+	7/7	17/17	P
Arginine	+	2/7	2/17	—
Lysine	+	4/7	5/17	—
Aminobutyric acid	Sometimes	2/7	5/17	P
Tyrosine	Sometimes	6/7	10/17	P?
Threonine	Sometimes	3/7	14/17	P?
Glycine	+	7/7	17/17	P
Serine	+	6/7	14/17	P
Glutamic acid	+	7/7	17/17	P
Taurine	Sometimes	0/7	0/17	?
Aspartic acid	+	7/7	11/17	?
Phenylalanine	Sometimes	1/7	5/17	?
Glutamine	Sometimes	2/7	2/17	?
Other spots	Sometimes	4/7	11/17	?

* Symbols: P = present; P? = presence uncertain; ? = normally variable; and = not present.

We have not compared the amino acid content of the urine with the results obtained by chromatography. However, chromatograms of the blood and urine of 7 children suffering from kwashiorkor appear to follow a similar pattern.

In patients suffering from a definite form of kwashiorkor it would seem that, at first, the spots are more numerous and more intense than is the case with controls. Alanine, glycine, and serine appear to be constantly present in the urine, and leucine, valine, proline, tyrosine, and threonine are frequently found. Lysine, aspartic acid, and phenylalanine are not found when this technique is used. Tryptophan and methionine are broken down and probably account for a certain number of ill-defined spots.

Conclusion. Paper chromatography is not a sufficiently reliable test for the absence of or the reduction in quantity of a given amino acid. At the most, all one can say is that the simultaneous presence in the blood or in urine of a certain number of amino acids indicates that kwashiorkor cannot be related to their absence. Column chromatography would give more precise and useful results.

Thus, neither dietary investigation nor chromatographic research is able to give us precise information about the amino acid deficiencies related to kwashiorkor. The practical results of supplementing the basic diet with several amino acids should now be tried. All the evidence indicates that the limiting factor is lysine. It is therefore chiefly this amino acid that we used.

Study of the Direct Supplementation of Basic Diet by Amino Acids

The addition of amino acids to the basic diet was carried out in the following manner for testing purposes. Children of approximately the same age (several weeks after weaning) who showed unmistakable signs of kwashiorkor were hospitalized and maintained on an unbalanced basic diet consisting essentially of millet, potatoes, and preserves. This diet gave a sufficiently high intake of calories (900 to 1000).

First study. To 3 children, a preparation consisting of the following components was administered:

Tryptophan	1.4
Methionine	3.6
Lysine	5.0
Sodium bicarbonate	3.5
Sucrose	10.0
Distilled water (as needed)	300.0

which amounts to 10 gm. of DL amino acid administered in 2 lots of 150 gm. of the solution. This is administered by bottle or, if the child refuses the bottle, force feeding by tube is substituted. This, however, was not found to be a good procedure, since the mixture of the 3 amino acids has an unpleasant taste, and force feeding often resulted in vomiting. This technique has the additional disadvantage that it calls for a too-abrupt administration of the amino acids, whereas it would be better to spread this over the whole day.

Second study. In experiments carried out with 4 children, we gave DL-lysine in 4 doses of 1 gm. each, daily, mixed this time in broth or purée. This was well accepted and tolerated. It is difficult to evaluate the results observed.

Tests conducted with these children can never be very prolonged, since kwashiorkor never maintains any *status quo*, and even a slight case of the disease can become severe very rapidly. The periods of observation and of waiting must therefore be limited and strictly controlled.

Kwashiorkor patients have poor appetite and usually refuse to take all the food given to them in the basic diet. In order to stimulate appetite we have had to vary the appeal of the diet by adding different cereals, the amino acid content of which is mutually complementary, and our observed results in this way became distorted.

Often infection tips the nutritional balance, causing kwashiorkor and interfering with observations based solely on nutritional considerations. Because of the high cost of amino acids, tests are limited to a few patients only.

The effectiveness of this treatment is shown by the disappearance of edema, the disappearance of lesions of the skin and mucous membranes, a rise in the blood protein level, a return of the electrophoretic pattern to normal, and, finally, modifications in fatty liver deposits.

I shall summarize here what we can deduce from our results, which are set forth in detail in the APPENDIX.

First, results obtained in patients receiving 3 amino acids:

Case 56,108 (FIGURE 6). Typical kwashiorkor. For 7 days the child received varied cereals and lost 400 gm. The blood protein level rose from 4.3 to 5.8, and albumin increased from 1.7 to 2.0 gm. During the following 25 days a mixture of 3 amino acids was added to the basic diet and was quite well tolerated by the child. After 8 days, steatosis had regressed, and the blood protein level had risen to 6.8 with a rise in albumin from 2.0 to 2.75 gm. These values tended to decrease during the next few days, during which time the child ran a fever. The standard diet of milk plus hydrolyzate was then prescribed, and progress became rapid. The child was released as cured, having gained 1 kg. in 18 days. The protein level in the blood had increased from 7.0 gm. to 7.15 gm. and had finally reached 7.30 gm.

Case 56,443 (FIGURE 7). Typical kwashiorkor. After 2 days on a millet diet the child was given a mixture of amino acids for 4 days, but this was poorly tolerated. The general condition of the patient deteriorated, and the blood sugar level fell to 60 mg. The standard diet was then tried, and the protein level of the blood rose. However the child died 16 days later from a concurrent infection (otitis, lung abscess, and staphylococcal pericarditis).

Case 56,455 (FIGURE 8). Typical kwashiorkor. This patient lost 300 gm. in the first 3 days of hospitalization while on a mixed cereal diet. Amino acids were poorly tolerated. Since the patient's general condition had become poor, the blood sugar level falling to 55 mg., the standard diet was prescribed and a rapid cure resulted.

The first of these cases seemed to give promising results since protein level in the blood rose from 4.3 to 6.8 and the albumin level from 1.7 to 2.75; the amino acids were tolerated, although imperfectly, for 18 days.

We should note, however, that beginning with the first day of hospitalization, edema began to disappear, and improvement took place in spite of the very unbalanced diet administered to the child at that time. This could

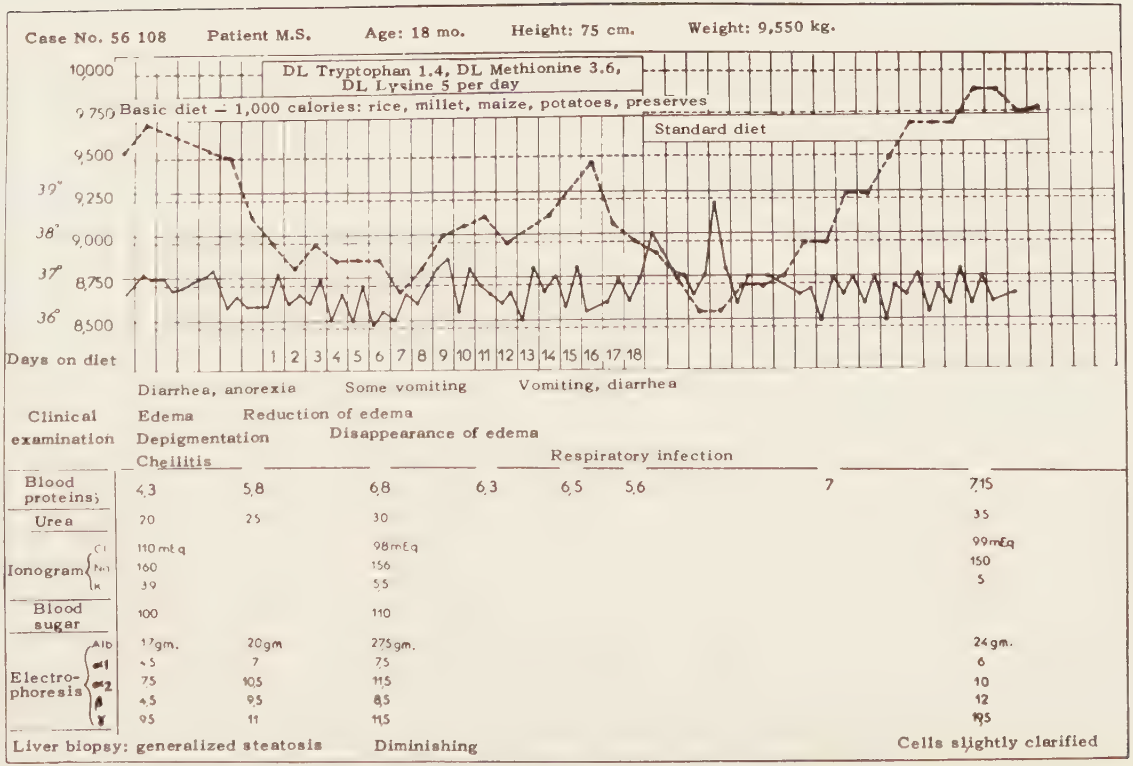


FIGURE 6. Record of Case 56,108 (patient M. S., aged 18 months).

result either from bed rest or from the more varied diet. In the other cases, the results were more pronounced, and we had to discontinue tests quite early because of the deterioration of the general condition. In the last case, especially, the impression we received was that our treatment caused an aggravation of the disease.

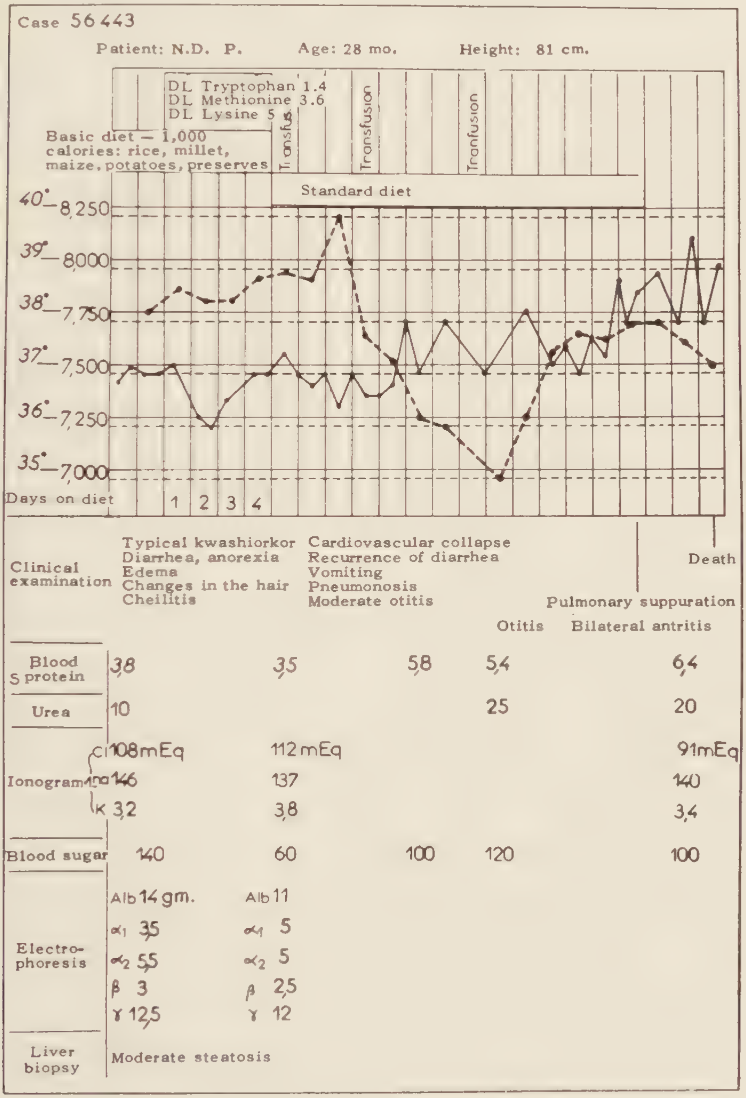


FIGURE 7. Record of Case 56,443 (patient N. D. P., aged 28 months).

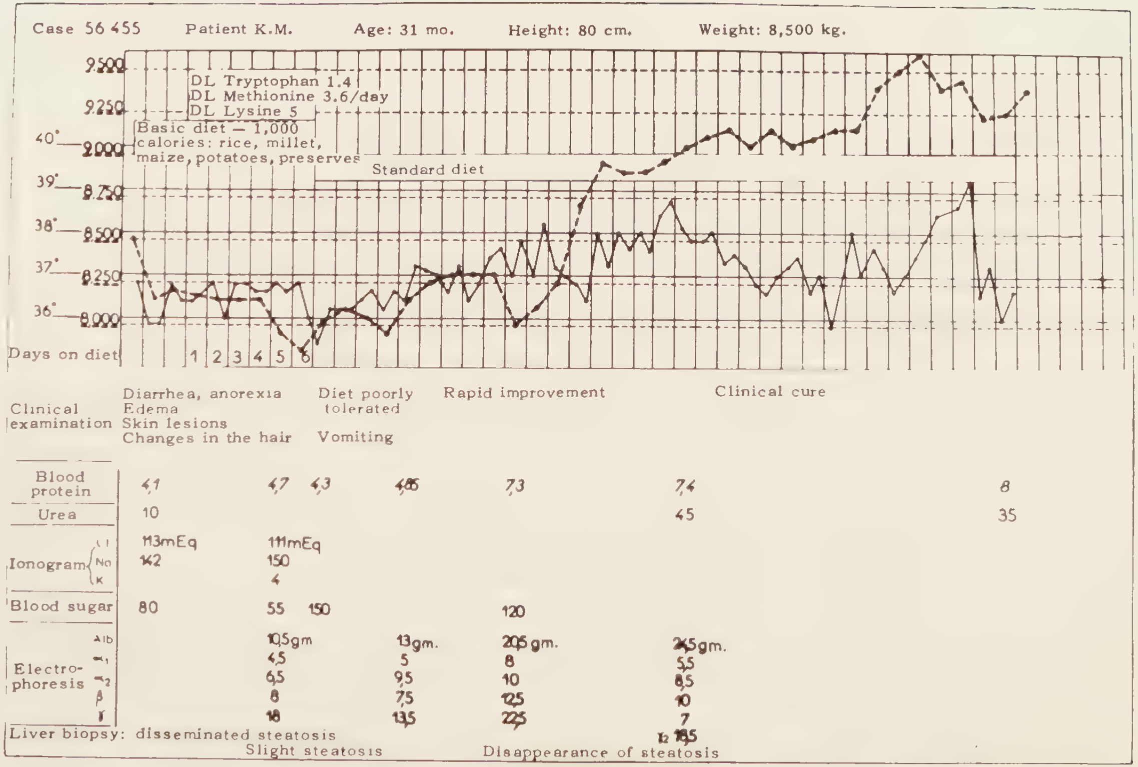
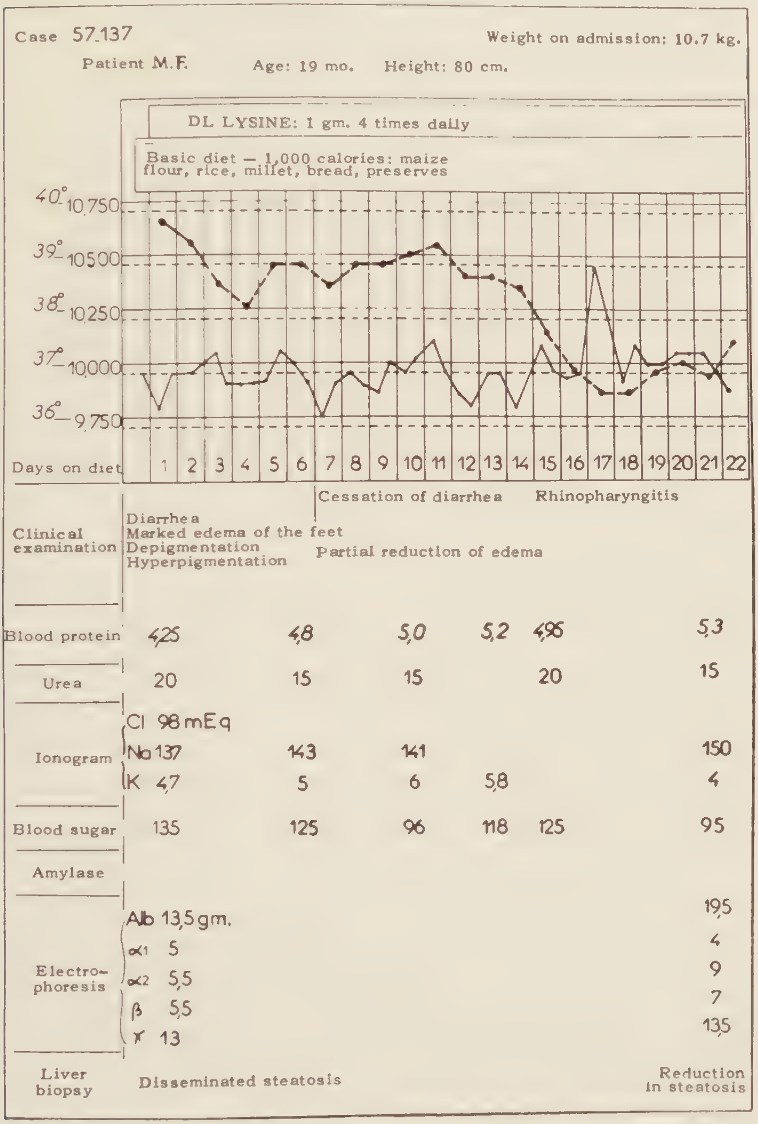


FIGURE 8. Record of Case 56,455 (patient K. M., aged 31 months). Typical kwashiorkor.

We resumed these tests later, but limited ourselves to the addition of lysine alone to the basic diet. We chose 4 patients with medium kwashiorkor for these tests, and I summarize our results as follows:

Case 57,137 (FIGURE 9). Blood protein level 4.25, presence of steatosis.



Clinical examination	Diarrhea Marked edema of the feet Depigmentation Hyperpigmentation	Cessation of diarrhea Partial reduction of edema			Rhinopharyngitis	
Blood protein	4,25	4,8	5,0	5,2	4,95	5,3
Urea	20	15	15		20	15
Ionogram	Cl 98 mEq Na 137 K 4,7	143 5	141 6	5,8		150 4
Blood sugar	135	125	96	118	125	95
Amylase						
Electrophoresis	Alb 13,5 gm. α1 5 α2 5,5 β 5,5 γ 13					19,5 4 9 7 13,5
Liver biopsy	Disseminated steatosis					Reduction in steatosis

FIGURE 9. Record of Case 57,137 (patient M. F., aged 19 months).

Slow increase in blood protein level, interrupted on the fifteenth day by infection. Almost entire regression of steatosis by the twenty-first day.

Case 57,138 (FIGURE 10). Blood protein level 4.4, considerable steatosis. On application of the treatment, diarrhea ceased, edema disappeared (a decrease of 1000 gm. in 1 week), and the weight curve rose slightly. The attitude

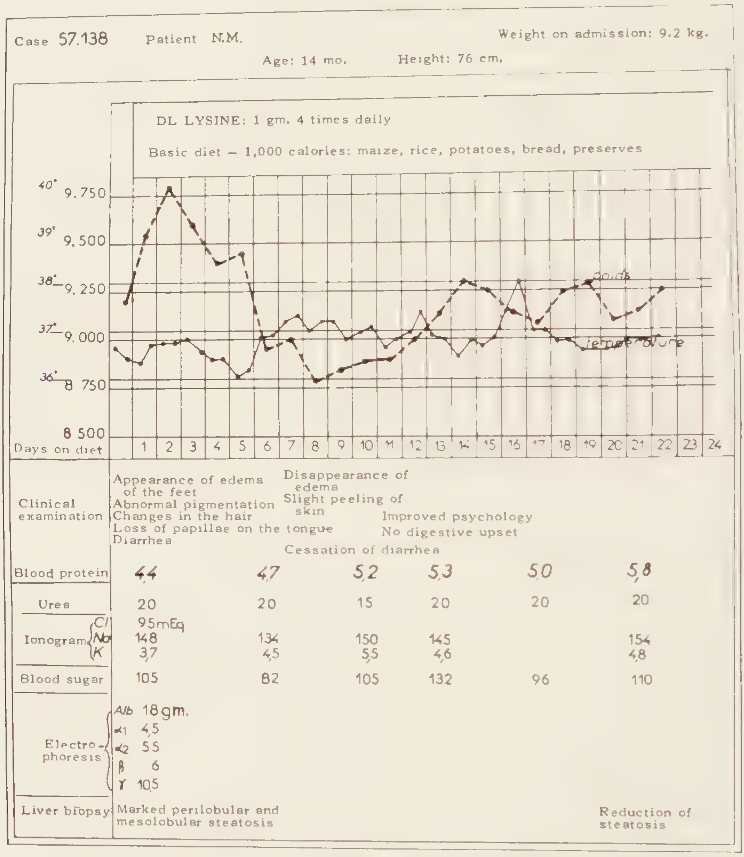
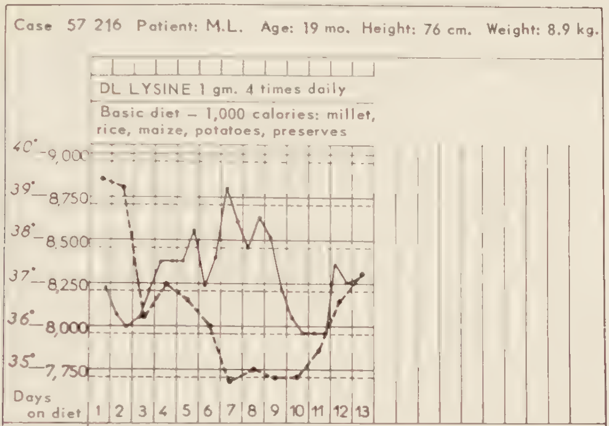


FIGURE 10. Record of Case 57,138 (patient N. M., aged 14 months).

of the child improved. Blood protein rose from 4.4 to 5.8 on the twenty-first day of treatment, and steatosis regressed considerably.

Case 57,216 (FIGURE 11). The diet brought about loss of weight of 1 kg. 300 gm. in 7 days. Concurrent pulmonary infection was observed. The blood protein level rose from 4.6 through 5.2 to 5.6 by the tenth day. Steatosis, very marked on admission, was still apparent in lessening degree on the tenth day.

Case 57,119 (FIGURE 12). Kwashiorkor with widespread steatosis. The blood protein level was 4.0. After 3 days of diet and hospitalization the weight fell by 1350 gm. and the blood protein level rose from 4.0 to 5.2. After 4 days on lysine the blood protein level remained at 4.7. The child was then taken back by the family, and the blood protein level 5 days after release was 5.5.



Clinical examination		Diarrhea Anorexia Edema Depigmentation	Regression of edema		
Blood protein		4,6	5,2	5,6	
Urea		20	20	20	
Ionogr.	Cl	95 mEq			
	Na	143		146	
	K	4		4,3	
Blood sugar		0,95	1,08		
Electrophoresis	Alb	1,95 gm.			
	α1	4			
	α2	8			
	β	5,5			
	γ	15			
Liver biopsy		Widespread steatosis			Steatosis still marked

FIGURE 11. Record of Case 57,216 (patient M. L., aged 19 months).

These results seem to show that the addition of 4 gm. of DL lysine per day is well tolerated, leads to a definite improvement, proves capable of increasing the level of blood protein, and causes regression of steatosis, although these results are obtained rather slowly. On the other hand, we should note that in Case 57,119 (FIGURE 12) hospitalization and bed rest more than diet, which was partially refused, brought about considerable reduction in edema.

These tests should be continued with varying quantities of lysine, and the diet should be supplemented with other amino acids.

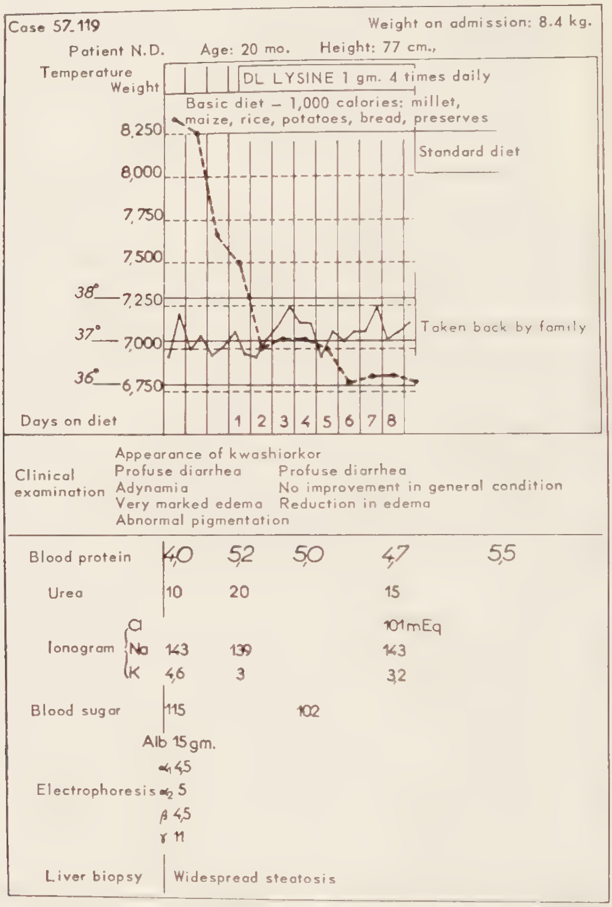


FIGURE 12. Record of Case 57,119 (patient N. D., aged 20 months).

CONCLUSION

This report has presented results obtained in the treatment of kwashiorkor at Dakar, French West Africa, without mentioning the very interesting work carried on in other countries, especially on amino acids, by Holt[34] in New York and Brock and Hansen[36] in South Africa.

In curative treatment, protein hydrolyzates appear to give quicker results than does whole protein, and it is necessary to add potassium to the diet. The prolongation of a high-protein diet seems to be beneficial and without danger.

In preventive treatment, a mixture of good biological value is obtained when the basic component, millet, is supplemented with peanut press cake and fish flour.

The addition of amino acids, singly or in groups, seems to show that lysine has a limited effect.

We think that for the present the amino acid metabolism is too little known to enable us to determine the specific deficiency involved in kwashiorkor. The attainment of knowledge in this connection will be the objective of workers in the future. On the other hand, we are of the opinion that use of the mixed diet (that is, of carefully prepared mixtures of local products low in price) is very effective and can produce results within a short period. Experiments with those products, since they are more difficult with human subjects than with animals, will bring about improvement in the nutrition of the peoples of the underdeveloped areas of the world.

Acknowledgments

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Appendix

Case 56,108 (figure 6). Patient M. S., 18 months, 9.55 kg., 75 cm.

Admitted January 18, 1956 (one month after weaning) with diarrhea, general edema, loss of pigmentation, and cheilitis.

Protein, 4.3 gm. per cent; urea, 20 mg.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	39.5	17.0
α_1	10	4.5
α_2	18	7.5
β	10	4.5
γ	23	9.5

A/G = 0.65

Freezing point depression, corrected, −0.58° ; Cl, 110 mEq.; Na, 160 mEq.; K, 3.9 mEq.

Blood sugar	100 mg. per cent	
Blood count	5,050,000	(hemoglobin 80 per cent)
Leukocytes	10,700	
Malaria	negative	

Liver biopsy: general steatosis in single vacuoles.

The child was kept under observation for 7 days. A diet made up of millet soup, corn flour, rice, potatoes, and preserves was given. The child lost 400 gm. Blood protein level rose to 5.8 from 4.3. This improvement, which was achieved despite the very unbalanced diet, can be attributed either to bed rest or to the fact that this diet was more varied than that received at home.

Biological tests given on January 24:

	Per cent	Gm./liter
Total proteins 5.8		
Albumin	34.5	20.0
α_1	10.5	7.0
α_2	18.5	10.5
β	16.0	9.5
γ	20.5	11.0

January 24 to February 18, same basic diet, plus following mixture (force fed):

Tryptophan	1.4
Methionine	3.6
Lysine	5.0

The child, however, tolerated this mixture rather poorly and occasionally vomited. General condition remained fairly satisfactory, however, and the child was not too exhausted.

January 30 (sixth day on diet):

Proteins	6.8
Blood urea	30 mg.

Cl, 98 mEq.; Na, 156.5 mEq.; K, 5.5 mEq.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	41.5	27.5
α_1	11.0	7.5
α_2	17.5	11.5
β	12.5	8.5
γ	17.5	11.5

Liver biopsy: regression of steatosis.

During the following days, the child continued to vomit frequently during or after force feeding.

The level of protein in the blood did not improve and even fell a little, as follows:

Date	Blood protein
January 30	6.8
February 4	6.3
February 8	6.5
February 11	5.6

The weight curve fell, and there was vomiting and diarrhea. It should be noted that at that time the child showed considerable inflammation of the nasopharynx accompanying teething.

February 11. Administration of the mixture of the 3 selected amino acids was stopped, and the standard diet (milk and protein hydrolyzate) was given.

The child's condition remained stationary, and loss of weight continued for several days. A rapid improvement was then observed.

Blood protein level rose 7.00 per cent (February 20), 7.15 (February 27).

A fourth electrophoretic analysis indicated the following:

	Per cent	Gm./liter
Albumin	33.5	24.0
α_1	8.5	6.0
α_2	14.0	10.0
β	16.5	12.0
γ	27.5	19.5

A/G = 0.5

February 27. Third liver biopsy: the cells of the liver were still somewhat clarified.

February 11 to February 29: The child's condition improved daily, and the weight rose from 8.8 to 9.8 kg.

The child was released on February 29, and was examined again at consultation on April 16. At that time he was in a good state of nutrition. The blood protein level was 7.5.

Case 56,443 (FIGURE 7). Patient N'D. P., 28 months.

Typical kwashiorkor: diarrhea, significant edema, loss of pigment on the face and groin. Hair lank, thin, and pulled out easily.

Cheilitis, smooth tongue, tendency to collapse.

Blood protein level, 3.8; urea, 10 mg.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	35.5	14.0
α_1	9.5	3.5
α_2	14.5	5.5
β	9.0	3.0
γ	31.5	12.5

Freezing point depression, corrected, −0.57°.

Cl, 108 mEq.; Na, 146 mEq.; K, 3.2 mEq.; blood sugar, 140 mg ; blood amylase, 50 units.

Blood count and composition were normal.

Coproculture: colonies of pathogenic staphylococci.

Liver biopsy: steatosis of portal vein and medial lobes.

For the first 2 days the child was given a mixed cereal diet. For the 4 following days, the child was given this basic diet plus a mixture consisting of tryptophan, methionine, and lysine. This child was forcibly fed.

The child tolerated the first 2 force feedings well, but vomited on the second and third days. Weight remained stationary, and the general condition declined.

Blood protein level, 3.5; urea, 20.0 mg.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	31.0	11.0
α_1	14.5	5.0
α_2	14.0	5.0
β	7.0	2.5
γ	33.5	12.0

Cl, 112 mEq.; Na, 137 mEq.; K, 3.8 mEq.
Blood sugar lower (60 mg.).

After this 4-day trial, the child was put back on the standard diet. Five days later the blood protein level was 5.8 mg. and the blood sugar level was 100 mg.

However, infectious complications developed. There was otitis of both ears, diarrhea, and abscess of the lung. Leukocytes rose to 39,300, and in spite of the use of antibiotics (chloramphenicol and erythromycin) and plasma transfusions, the child died 16 days after the start of standard treatment. The autopsy confirmed the presence of lesions in the ear and lung and revealed pericarditis of staphylococcal origin.

There were also evidences of infection and congestion of the liver.

The pancreas was very unusual and showed alternating areas of normal tissue and very compact inflamed cirrhotic tissue.

Conclusion. After poorly tolerating the injection of amino acids, this typical kwashiorkor patient died later of ancillary infection.

Case 56,455 (FIGURE 8). Patient K. M., 31 months, 80 cm., 8.5 kg.

Five months after weaning, this child was recovering from measles and was suffering from diarrhea, edema of the hands, feet, and face, loss of pigmentation in the area of the groin, hair sparse, faded, and pulled out easily. There was no cheilitis, and the tongue was normal.

Blood protein level, 4.1; urea, 10 mg.
Blood sugar level, 80 mg.
Blood amylase, 75 units
Freezing point depression, corrected, −0.57°
Cl, 113; Na, 142
Red cells, 2,830,000

Liver biopsy: considerably widespread steatosis.

The child was hospitalized, put under observation, and given a diet consisting of cereals alone for 3 days. There was a weight loss of 300 gm.

For the next 6 days, a mixture of 3 amino acids was added to the same basic cereal diet. This mixture was poorly tolerated and caused vomiting.

On the fourth day of this diet: blood protein, 4.75 per cent; A/G, 0.29.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	22.5	10.5
α_1	9.5	4.5
α_2	13.5	6.5
β	17.0	8.0
γ	37.5	18.0

Blood sugar level, 55 mg.

On the fifth day the child's general condition was poor, and his reactions were apathetic. The standard diet (skim milk plus protein hydrolyzate) was then given.

Rapid improvement:
On fourth day of treatment: blood sugar, 80 mg.; urea, 15 mg.
On fifth day of treatment: blood protein level, 4.85.

Electrophoretic analysis:

	Per cent	Gm./liter.
Albumin	27.0	13.0
α_1	10.0	5.0
α_2	19.5	9.5
β	15.5	7.5
γ	28.0	13.5

On the tenth day: blood protein, 7.3; blood sugar, 120 mg.; blood amylase, 210 units.
On the eleventh day: liver biopsy—disappearance of steatosis.
On the eighteenth day: blood protein level, 7.4; urea, 45 mg

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	33.0	24.5
α_1	7.5	5.5
α_2	11.5	8.5
β	13.5	10.0
γ	9.5	

Reactions normal, good appetite. Weight rose by 1.15 kg. and skin lesions disappeared.

On day 33: blood protein level, 8.1 per cent; urea, 35 mg.

The child was then released from hospital. Examined one month later and found to be in excellent health.

Conclusions. Typical kwashiorkor. Loss of weight at the time of hospitalization and during feeding of mixed cereal diet. Amino acids poorly tolerated. Rapid cure on standard diet.

Case 57,216 (FIGURE 11). Patient M. L., 19 months, 76 cm., 8.9 kg.

Ill since 1 month after weaning, with diarrhea, anorexia, and edema of the lower limbs and of the face. Slight depigmentation of face and lower limbs. Hair lank and pulled out easily. No cheilitis.

Cereal diet plus DL lysine, 1 gm. 4 times daily. Loss of weight (1.3 kg. in 7 days), disappearance of edema, rise in temperature following acute pulmonary infection.

Blood protein level: 4.6 on admission
5.2 on day 2
5.6 on day 10
Liver biopsy: widespread steatosis at admission.
Day 10: steatosis still marked.

Case 57,119 (FIGURE 12). Patient N. D., 20 months, 77 cm., 8.4 kg.

Two months after weaning, with diarrhea, anorexia, and adynamia. Generalized edema. Depigmentation of the cheek. Hyperpigmentation in region of buttocks. No cheilitis.

Liver biopsy: generalized steatosis.

Hospitalization. Cereal-based diet administered for 3 days and refused by the child. Loss in weight (1.35 kg.), disappearance of edema.

Addition of DL lysine—1 gm. 4 times daily.

Change in blood protein level: 4.0 to 5.2 before administration of lysine; 5.0 to 4.7 after 4 days on lysine.

Child taken back by its family, given 2 injections of plasma and followed at consultation.

Blood protein level (4 days after release), 5.5.

Case 57,137 (FIGURE 9). Patient M. F., 19 months, 80 cm., 10.7 kg.

Two months after weaning, with vomiting, diarrhea, and edema of 10-day duration. Inflammation of the nasopharynx. Typical moderate kwashiorkor. Edema of the feet and depigmentation in the region of the cheek. Area of hyperpigmentation with peeling of skin. No cheilitis, no change in the hair.

Protein, 4.25; urea, 20 mg.; blood sugar, 135 mg.

Ionogram: Cl, 98; Na, 137; K, 4.7 mEq.

Diet was 1 gm. of DL-lysine administered 4 times daily in addition to the basic diet of corn, rice, millet, bread, and preserves.

This diet was well tolerated. On disappearance of edema, the weight fell by 500 gm. in 4 days, remained stationary, and then fell again on the fourteenth day, following an infection. Diarrhea ceased on the sixth day. The patient's appetite was good, and his general condition was unchanged.

Change in blood protein level:
4.25 on admission   4.8 on day 6   5.0 on day 10
5.20 on day 13   4.95 on day 15 (infection)   5.30 on day 31

Electrophoretic analysis:

	On admission		Day 21	
	Per cent	Gm./liter	Per cent	Gm./liter
Albumin	31.0	13.5	36.0	19.5
α_1	11.5	5.0	8.0	4.0
α_2	13.5	5.5	16.5	9.0
β	13.0	5.5	14.0	7.0
γ	30.5	13.0	25.5	13.5

Liver biopsy on admission: multiple vascular steatosis disseminated throughout the organ. On day 21 of treatment: almost total regression of steatosis.

Case 57,138 (FIGURE 10). Patient N. M., 14 months, 76 cm., 9.2 kg.

One-and-one-half months after weaning. Diarrhea of 3-week duration, edema of the feet, skin dry and brittle on the legs, area of hyperchroma and peeling of the skin on the thigh, hair pulled out easily. No cheilitis.

Proteins, 4.4; urea, 20 mg.

Electrophoretic analysis:

	Per cent	Gm./liter
Albumin	40.0	18.0
α_1	10.5	4.5
α_2	12.5	5.5
β	13.6	6.0
γ	24.0	10.5

Ionogram: Cl, 95; Na, 148; K, 3.7.

Blood sugar level, 105 mg.

Liver biopsy, marked perilobular and mesolobular steatosis, inflamed portal fibrosis.

Standard diet: cereals and preserves plus DL-lysine, 1 gm. 4 times daily.

Development: no digestive upsets, good appetite, cessation of diarrhea, disappearance of edema (1000 gm. in one week), slight peeling of the skin.

The weight curve then rose slightly. The child's behavior improved, and he became gay and vivacious.

The blood protein level then went from 4.4 on admission to 5.2, 5.3, 5.0, and finally reached 5.8 on day 21 of treatment. The steatosis regressed.

PRINCIPLES OF TREATMENT AND PREVENTION OF SEVERE PROTEIN MALNUTRITION IN CHILDREN (KWASHIORKOR)*

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It is now generally recognized that kwashiorkor is the result of a severe deficiency of dietary protein at an age when the essential amino acids are required in relatively large quantities for growth. In most children developing kwashiorkor the protein intake is deficient, not only in quantity, but also in quality. Thus, the diets that are responsible for the syndrome usually contain protein that, due to its low biological value, is poorly utilized.

If protein deficiency occurs in children who are receiving an adequate or even excessive quantity of calories, as has been reported in Jamaica,[1] the resulting clinical picture is that of classic kwashiorkor. This form is characterized by edema, lesions of the skin, hair changes, apathy, anorexia, enlarged fatty liver, and low serum protein. These children also have abundant subcutaneous fat and recover rapidly with a high protein diet; when they loose their edema there is no wasting evident.

In the majority of the regions of the world in which kwashiorkor is found, however, children after weaning receive diets that are deficient not only in protein but also in calories. The clinical picture differs from that of classic kwashiorkor in that the children often show a considerable degree of tissue wasting, and the response to treatment is much slower. They are characterized by a reduction in statural growth and in weight, marked diminution or even absence of subcutaneous fat, and a much greater degree of muscular wasting; the liver, although it shows fatty change, is usually not increased in size. Since these children have a marasmic appearance when their edema is lost, this form of malnutrition is spoken of as marasmic kwashiorkor.

The diets of children developing kwashiorkor are not only low in protein, but are also deficient in other essential nutrients, particularly in the marasmic form of the disease. For this reason and also because their absorption is reduced,[2, 3] a deficiency of fat-soluble vitamins is a particularly common accompaniment of kwashiorkor.[4] Signs suggestive of B-complex vitamin and iron deficiencies are also frequently found associated.[5-7] Due largely to

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variations in the customary diet, the relative importance of these various vitamin and mineral deficiencies that complicate the clinical appearance vary greatly.

Our concept of the various types of kwashiorkor and their relationship to marasmus is presented graphically (FIGURE 1). This chart shows a continuous range of types between marasmus, which is due basically to a severe lack of food, but with a normal protein-to-calorie ratio, and kwashiorkor, which is basically produced by a deficiency of protein combined with almost any other degree of caloric intake. The body of the pyramid indicates the degree of severity, which is also a continuous transition from the well-nourished child (represented by the apex) to the most severe form of any of the types of malnutrition mentioned. In the intermediate part of the pyramid are represented the cases of prekwashiorkor and the other mild forms of protein deficiency which occur in a high percentage of children in underdeveloped areas. The closer to the apex of this pyramid, the less pronounced become the signs and symptoms and the narrower becomes the degree of differentiation between the various types.

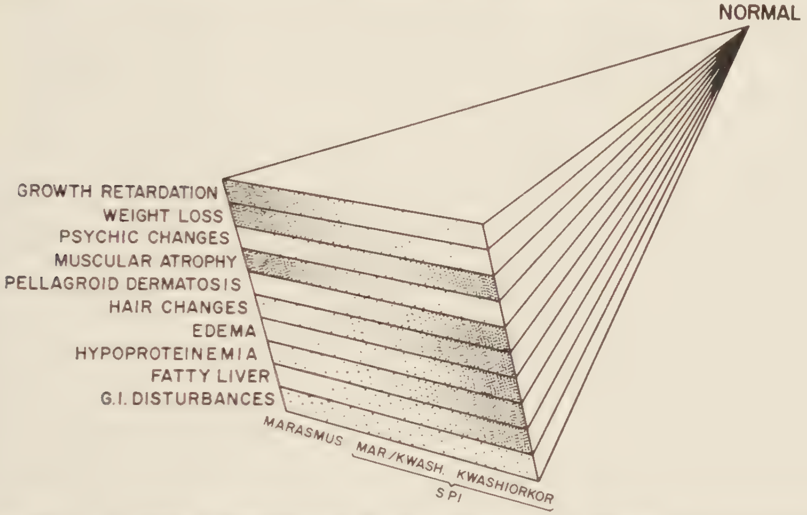

FIGURE 1. Schematic representation of the interrelation of kwashiorkor and marasmus. At the left of the pyramid base the intensity of the stippling suggests the frequency with which the signs and symptoms listed appear in marasmus. At the right the occurrence of the same signs and symptoms in "classic" kwashiorkor are portrayed. In between are all combinations between these two extremes. The term *síndrome pluricarencial de la infancia* (S.P.I.) is used in Latin America to cover both "classic" and marasmic kwashiorkor; the great majority of the cases are of the latter type. The severity of the signs and symptoms is indicated by the distance from the apex. Reproduced by permission of the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, C. A.

This diagram is not merely theoretical, since the cases encountered under field conditions show every conceivable variation in signs and symptoms as represented in the diagram. It should be emphasized, however, that when the extreme of partial or complete starvation is reached (marasmus), the signs of specific protein deficiency are no more manifest than are those of other specific nutrients, all of which are, of course, grossly deficient.

Although many cases gradually develop kwashiorkor due to the inadequacy of the diet alone, in most cases other factors operate as contributory or precipitating causes.[8] These help to determine the relatively small number of children who will develop the clinical syndrome in a population in which the majority are fed on inadequate diets. Among these factors, acute and chronic infections are important. In Central America, at least, the most common of the infections that serve to precipitate kwashiorkor is diarrhea of infectious origin. Other infections that are likely to act similarly are malaria and the contagious diseases of childhood. Socioeconomic factors such as the death of a parent, abandonment, or loss of work by the head of the family are also frequently involved.

We have recently observed a child who developed diarrhea while on repeated metabolic balance trials with milk feeding. With moderate diarrhea of short duration, this child showed a markedly reduced absorption of nitrogen and a negative balance.[8] Diarrhea not only limits the absorption of nitrogen, but also considerably increases its excretion, so that the net retention drops. The situation is made even worse by the common and unfortunate practice of withdrawing solid food from the child who develops diarrhea and substituting starch gruels. Educational efforts should be aimed, not only at the correction of diets given to children with diarrhea, but also at avoiding the dangerous practice of using laxatives or purgatives in the treatment.

Principles of Dietary Treatment

There is no doubt that the administration of a diet rich in proteins of high nutritive value is an effective treatment of kwashiorkor.[9-12] The majority of authors recommend the use of either half-skim or skim milk, particularly because of the proportionately high content of protein of such food. Some consider also that the lower fat content, as compared with that of whole milk, is beneficial because the capacity of the sick child to digest fat is seriously impaired. However, Gómez obtained equally satisfactory results using whole milk,[13] and Dean has recently recommended the addition of 30 to 50 gm. of fat to diets for the treatment of kwashiorkor.[14] The conclusion that a normal amount of fat in the diet is not harmful is strongly supported by the work of Holt in children with diarrhea from other causes.[15] Although both our own studies[16] and those of Gómez[17] demonstrate that fat is poorly absorbed during the first few days of treatment, the amount absorbed is proportional to the quantity of fat ingested, and the child soon recovers most of his ability to use dietary fat.

The profound anorexia that is characteristic of children with kwashiorkor and the reduced activity of the digestive enzymes[18-20] make it necessary to begin treatment with relatively small amounts of protein, generally in the range of 2 gm./kg. The prompt recovery of enzyme activity[20] and returning appetite make it possible to increase this rapidly to values in the range of

5 gm. kg. at the end of the first week or during the second. This intake can later be increased still further in order to correct the protein depletion as rapidly as possible. Nitrogen balance studies indicate clearly that, within a broad range, nitrogen retention in kwashiorkor does increase with a rising intake, although the percentage of nitrogen retained out of the total absorbed will drop, of course, as the protein intake increases[21, 22] or as nitrogen stores are gradually repleted.[16]

It is very important that the caloric intake be sufficiently high to avoid the use of protein by the body to supply energy and, as mentioned previously, this is the rationale behind the use of added fat in therapeutic diets.[14] There have been reports of the poor tolerance of sugars, especially sucrose and lactose[23, 24] and, for this reason, the use of bananas as a calorie source has been suggested. There is no objection to the use of bananas, since they are well tolerated and are an excellent calorie source. The reports of intolerance to sugars are based, however, only on clinical observations and need further study. As soon as appetite and tolerance permit, the child should be given fruit juices, green and yellow vegetables, meat, eggs, and other foods necessary to give the balanced diet required for complete recovery.

Vitamin and Mineral Supplements

With an initial diet of milk alone, there occurs a prompt recovery from all of the signs and symptoms of kwashiorkor, including those that might be attributed to a lack of vitamins of the B complex. It is not only unnecessary to use therapeutic vitamin preparations; the use of such preparations may actually be harmful. In the experience of Magalhaes Carvalho,[25] for example, the administration of high doses of vitamin B complex increases the mortality from kwashiorkor, a phenomenon that Frontali[26] explains as due to the greater demand for labile methyl groups for the transformation of nicotinic acid into trigonellin. The greater deficiency of methyl groups may in turn aggravate the liver damage.

There is no demonstrated benefit from the use of either folic acid or vitamin B_{12}, since the anemia, although frequently macrocytic, responds satisfactorily to milk alone, unless there exists a marked iron deficiency.[3] Despite the fact that the serum levels of vitamin C tend to be low, at least in Central American cases,[27] the deficiency does not reach the point where obvious clinical signs are observed, and it is a simple matter to add fruit juice and other natural ascorbic acid sources to the diet.

We do consider justifiable, however, the addition of moderate doses of vitamin A from the beginning of treatment, especially when half-skim or skim milk is used. Partially defatted milk does not correct the deficiency of vitamin A, which has been amply confirmed by studies of the vitamin A content of blood serum and liver tissue,[3] and which may even be so severe as to cause ocular lesions.

The majority of children show only a mild anemia, which is usually normocytic or slightly macrocytic in character. Even in uncomplicated cases, however, there is a tendency for the serum iron values to be low and for microcytosis to develop with dietary treatment unless supplementary iron is given. Accordingly, the relatively early addition of iron to help fill depleted iron stores is recommended.

Other Therapeutic Procedures

(1) *Blood transfusions.* In general, blood transfusions have no advantages over proper diet therapy alone. Although blood transfusions for the treatment of kwashiorkor were introduced many years ago[28] and are still used and recommended today,[29] our own experience demonstrates that they are rarely necessary. It is still desirable to employ them, however, in the occasional very severe case where the child is in imminent danger of death due to collapse and shock and in cases with very severe anemia.

(2) *Plasma and protein hydrolyzates.* The parenteral administration of plasma or proprietary protein hydrolyzates, which has often been recommended to combat the hypoproteinemia of kwashiorkor, does not appear to be justified. There is abundant evidence from the protein balance studies of Gómez[22] and Holemans and Lambrechts,[21] as well as from our own unpublished work, that protein given orally is sufficiently absorbed and well retained even at the beginning of treatment.

(3) *Antibiotics and chemotherapy.* In general, we favor the routine use of an antibiotic such as penicillin during the first 8 to 10 days of hospitalization, since secondary infections are very frequent. Bronchopneumonia is particularly common and may be asymptomatic and therefore not recognized clinically. This complication is found at autopsy to be a frequent cause of death during the early days of hospitalization.[30, 31] When a secondary infection can be definitely diagnosed, it is important that the treatment be initiated promptly to avoid the stress which may interfere with or even prevent recovery. Similarly, malaria and amebiasis should be treated as soon as diagnosed.

Intestinal parasitism due to helminths ordinarily should not be treated until recovery is well advanced. The child with the kwashiorkor syndrome is not well able to tolerate the type of treatment required for parasite eradication, and the presence of intestinal helminths does not appear to interfere significantly with recovery.

(4) *Electrolyte solutions.* Either in the immediate history of the child or during the first few days of hospitalization, diarrhea is an almost constant feature of the syndrome, and vomiting is frequent. Dehydration, therefore, is often severe, even in the presence of edema. This accentuates electrolyte imbalance. Potassium deficiency is likely to be particularly severe, since it is aggravated by both the diarrhea and vomiting as well as by the protein deficiency.[32-34]

Failure to correct electrolyte disturbances is now believed to be the most frequent cause of death during the first 48 to 72 hours of hospitalization,[34] and their correction should be one of the first therapeutic measures taken. In cases that are only moderately severe, the oral administration of appropriate electrolyte solutions gives good results, but in severe cases it is necessary to resort to parenteral administration. The problem of electrolyte therapy has received particular attention in the work of Gómez *et al.*[32] and is discussed in more detail by Gómez elsewhere in this monograph.[35]

(5) *Enzymes and lipotropic substances.* While it is true that there is a marked deficiency in the action of duodenal enzymes at the time of admission, they recover very rapidly with the initiation of dietary treatment, as shown by Véghelyi[20] and confirmed by our own observations on the prompt return of the child's ability to absorb both the nitrogen and fat in either milk or vegetable mixtures. The use of special enzyme preparations appears, therefore, to be unnecessary.

The same can be said for the various preparations with lipotropic activity that have been used because of the frequency and severity of fatty infiltration of the liver;[36, 37] this disorder is promptly and completely corrected by the feeding of protein of high biological value.

Response to Amino Acid Mixtures

It would be helpful to know whether kwashiorkor is produced primarily by an over-all deficiency of protein or whether it is associated with imbalances in individual amino acids such as occur when the small amount of protein that the child receives is derived largely from a single food of vegetable origin (as is the case of Central American children developing kwashiorkor on a predominantly corn diet). The fact that occasional cases are encountered in which the child has received its protein from mother's milk, although in grossly inadequate quantities, suggests that it is not amino acid imbalance per se.

An important contribution made by the South African group[38, 39] has shown that a clear-cut improvement of all of the major signs of kwashiorkor can be brought about by vitamin-free casein or a mixture of synthetic amino acids. The improvement occurs not only in the anorexia, apathy, cutaneous lesions, and edema, but also in serum protein, and results in what they designate as a satisfactory "initiation of cure."

Our own experience with amino acid mixtures* is limited to a single individual to whom a mixture of 18 synthetic amino acids similar to that used by Hansen *et al.*[39] was given for a period of 18 days. This treatment resulted in the "initiation of cure" as defined by these workers, that is, the edema and skin lesions began to disappear relatively soon and serum protein increased. The child remained apathetic and anorexic, however, and there was not the same degree of recovery in serum amylase, and in the pseudocholinesterase previously observed in cases treated with either milk[12] or a vegetable protein mixture.[10] As will be seen in TABLE 1, there was a decrease in the serum levels of all of the vitamins except vitamin A, which apparently was mobilized from tissue stores.

It does not invalidate the concept that kwashiorkor is due to protein deficiency to emphasize that no experimental treatment that fails to provide the necessary vitamins can be expected to be successful for more than a relatively short initial period. All that we know of nutrition in general, and of vitamin metabolism in particular, indicates that under these circumstances vitamin deficiencies, if not limiting initially, will become so later as a result of inadequate intake, especially when body stores are depleted as in the case of kwashiorkor. For this reason, the objection that the results obtained with synthetic amino acid mixtures are limited only to an "initiation of cure" is meaningless.

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* This work is being carried out in collaboration with Eugene E. Howe, from the Merck Sharp & Dohme Research Laboratories, Philadelphia, Pa., who prepared the amino acid mixture.

The results show that protein administration must be the central point of any rational therapy even though appropriate amounts of all other nutrients should obviously be given for best therapeutic results. They also lend added emphasis to the administration of vitamins in "physiological" rather than so-called "therapeutic" doses. As these studies are continued, more information of fundamental value in guiding therapy will undoubtedly be obtained.

TABLE 1

BLOOD CONSTITUENTS AT ADMISSION WITH KWASHIORKOR AND FOLLOWING 18 DAYS TREATMENT WITH SYNTHETIC AMINO ACIDS (14-MONTH-OLD BOY)

Per 100 ml.*	On admission	After amino acid therapy	Per 100 ml.*	On admission	After amino acid therapy
Total protein (gm.)	4.12	5.73	Vitamin A (μg.)	6.4	21.0
B_1 in RBC (μg.)	8.8	4.8	Carotene (μg.)	1.0	3.0
Free B_2 (μg.)	0.0	0.0	Vitamin E (mg.)	0.64	0.02
Total B_2 (μg.)	1.61	1.25	Cholinesterase (U)†	0.21	0.46
B_2 in RBC (μg.)	24.6	17.2	Amylase (U)‡	6	0
Ascorbic acid (mg.)	0.22	0.10	Alk. phosphatase (mM/1/hr.)	5.85	3.70

* All determinations in serum except red-blood cell, thiamine, and riboflavin.
† Actually "pseudocholinesterase" measured in Michel units.
‡ Smith Roe units.

Dietary Measures for the Prevention of Kwashiorkor

(1) *Use of cow's milk.* There is no doubt that the consumption of adequate quantities of cow's milk following weaning will prevent kwashiorkor. Unfortunately, there are a number of factors that limit the consumption of cow's milk in areas where the kwashiorkor syndrome occurs. These factors are, principally, the inadequate production and high cost of milk. The insufficient production of milk is often due to poor animal management, nutritional deficiencies in the rations, poor control of ectoparasites and endoparasites, and inadequate facilities for the conservation and distribution of feedstuffs and of milk. In many regions these factors can be corrected and milk production greatly increased, but only through major long-term national efforts and considerable expense. In other regions the agricultural limitations may be such that the production of sufficient milk cannot be anticipated. Furthermore, under the circumstances of most underdeveloped areas, the permanent large-scale importation of milk is likely to be economically impracticable and undesirable.

The high cost of milk prevents its sufficient use by low-income families in areas where kwashiorkor is endemic. The problem of safe handling, including storage and distribution in both the dairy and the home is another major obstruction to the use of fresh fluid milk. The use of powdered milk has a number of practical advantages, and efforts that have been made to set up milk-drying plants in underdeveloped areas, wherever justified by potential milk production, are to be commended.

It must also be recognized that in many regions there are cultural objections to the use of animal milk.[11] Often such milk is unfamiliar to the people. This difficulty is more easily overcome than an acquired fear of milk, a phenomenon based on unfavorable experience with contaminated, adulterated, and poorly preserved milk that has been responsible for producing diarrhea. All of these considerations suggest that, especially in the near future, complete reliance cannot be placed on milk as a practical means of preventing kwashiorkor in most regions of the world in which it is a serious problem, despite the fact that, from a nutritional point of view, milk is ideal.

(2) *Use of animal protein.* The extensive use of such other sources of animal protein as meat and eggs also involves the serious problems of insufficient production, high cost, and prejudice against their use for feeding small children. It has been suggested that fish, especially in the form of industrial preparations of fish flour or similar products, can be of great value in infant feeding. While such products can undoubtedly contribute to the solution of the problem in a number of areas, not all countries have sufficient fish to make industrial development practicable. Fish flours would also encounter the same resistance observed to the introduction of any new food, especially one that is radically different from that commonly consumed in the region.

(3) *Use of plant protein.* In view of the difficulty of solving the problem of protein malnutrition by the increased production and use of animal products alone, the consideration of plant sources of protein becomes imperative. The excellent monograph by Dean on *Plant Proteins in Child Feeding*[12] has stimulated active work on the problem in a number of regions, and recently the specialized agencies of the United Nations concerned with nutrition, WHO, FAO, and UNICEF,* have cooperated in promoting these developments.

The major difficulty in the use of vegetable proteins is the relatively poor biological value of proteins from single plant sources. There are also the potential problems of an excess of crude fiber, low digestibility, and the presence of toxic or interfering substances. There is the further possibility that processing can introduce toxicity or lower nutritive value. There are times when processing will be beneficial, particularly in making certain of the essential amino acids more available, but the conditions must be controlled.

Despite all of these problems, an increased use of local proteins of vegetable origin appears to be the most promising means of preventing protein malnutrition in many areas of the world, at least under the conditions that now prevail. Information as to the amino acid content and digestibility of vegetable protein sources, although very incomplete, is still sufficient to enable suitable combinations to be planned for chemical and microbiological analysis and for animal trials of their biological value. More precise information about amino acid requirements would help, however, in combining vegetable proteins to give mixtures whose net protein value is relatively good. Such vegetable protein combinations have the advantage not only of being relatively cheap, but also of fitting the habitual diet patterns of young children in the majority of regions in which kwashiorkor is a problem.

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* World Health Organization, Food and Agriculture Organization, and the United Nations International Children's Emergency Fund.

It would be desirable to devise a product that could be prepared in the home from local raw materials. This is scarcely practicable, since special knowledge and skill are required to combine vegetable proteins in the right proportions, and since many of the cheapest and best protein sources cannot be used directly for food without special processing. We believe, therefore, that vegetable protein mixtures designed for the prevention of kwashiorkor must be manufactured from low-cost ingredients within the agricultural resources of each region.

In planning vegetable protein mixtures, it is necessary to take into consideration the following 8 factors: (1) the amino acid content of the individual ingredients and the final product, (2) the possible presence of toxic or interfering factors, (3) the need for obtaining exact specifications for each of the components, (4) the necessity of avoiding processes that damage the quality of the protein, (5) the desirability of using products of local origin, (6) the fact that the final product must be inexpensive and easily preserved, (7) the requirement that it may be easily prepared in the home as an infant food by mothers of low-income families, and (8) the demand that it must not run counter to existing dietary habits and prejudices.

Even after all of these requisites seem to be met, the product should not be recommended for commercial production and mass consumption until the 4 following biological trials have been carried out: (1) the testing of the product for toxicity in at least 2 species of animals, (2) the demonstration of the biological value of its protein by animal growth studies, (3) the testing of its acceptability and effectiveness in children under careful observation, and (4) field trials completed in selected communities or small population groups.

Although the primary objective is to provide a supplementary source of protein of good quality, it is desirable that a vegetable protein mixture contain, also, adequate quantities of the vitamins and minerals that are likely to be lacking in the usual diet. INCAP has attempted to follow these principles and procedures in developing vegetable mixtures for the prevention of protein malnutrition in infants and young children in Central America. The work has resulted in a mixture that soon will be ready for field trials. It contains:

Components	Percentage
Dried corn *masa*	50
Sesame meal	35
Cottonseed press cake	9
Torula yeast	3
Kikuyu leaf meal	3

The specifications for each of these ingredients have been recently published.[40] *Masa* is the dough prepared from lime-treated corn that is usually made into flat cakes and cooked to make tortillas. It is the basic food of the rural and poor urban populations of Mexico and Central America and is dried and powdered to form one half of the mixture. The sesame and cottonseed press cake, which may both be produced locally in considerable quantities and at low cost as a by-product of oil extraction, are rich in protein of relatively good quality, and their amino acids help to complement those of corn.

Fresh young kikuyu grass is dried to produce a meal that provides the major source of vitamin A activity in the mixture. It is important that this or a similar dehydrated leaf meal be added to provide provitamin A, since vitamin A deficiency is a serious problem in Central America, particularly in the preschool child. The torula yeast furnishes an inexpensive natural source of additional niacin, riboflavin, and thiamine.

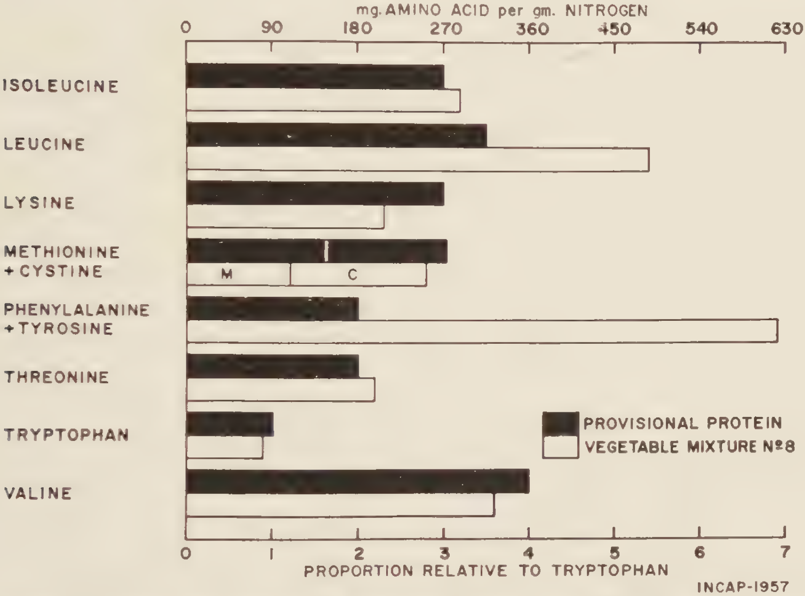

FIGURE 2. Amino acid pattern of provisional protein and INCAP Vegetable Mixture No. 8. Reproduced by permission of the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, C. A.

The degree to which the amino acid pattern of the mixture corresponds to that of the reference protein proposed by the FAO Committee on Protein Requirements[43] is shown in FIGURE 2. It will be seen that the mixture contains adequate quantities of most of the essential amino acids per gram of nitrogen. Small deficiencies by calculation of tryptophan, methionine, lysine, and valine are present. The magnitude of these deficiencies will vary somewhat with the particular corn used.[11, 15] The satisfactory degree to which the mixture would supply the estimated requirements for major vitamins and minerals,[46] if it were fed as the principal food and sole source of protein, is shown in TABLE 2. The mixture does not supply vitamin C since, in the countries of Central America, this vitamin is abundant in fruits and fruit juices that are available and can be given easily to the young child.

The mixture has thus far been fed to children only in the form of an *atole*, a purée, or a dessert. The *atole* is prepared by cooking the mixture in a double boiler with enough water to make a thin gruel and is flavored with sugar. The purée is prepared in the same way, except that less water is added, and salt and oleomargarine are used for flavoring. The dessert is essentially the purée flavored with sugar and vanilla and served cold. It is obvious that the mixture may be prepared and served in many other ways, and a precooked form is envisaged.

Tests in animals have shown that this mixture is of good protein quality for rats, and for baby chicks when the added requirement for lysine is met.[40] To date, 11 children have received this mixture. Among these is 1 who was fed the mixture for a period of 100 days as the sole source of protein and 4 others who have received it from the time of their admission with moderate-to-severe kwashiorkor. The results have been uniformly good, and no complications have been encountered. Furthermore, the intake and output of nitrogen was measured in 5 children receiving their dietary protein entirely from the vegetable mixture for 5 days. The results were then compared with similar periods immediately before or after in which the child received the same amount of protein from milk. The average absorption of the milk protein was 81 per cent, and that of the vegetable protein, 73 per cent. The average retention of the total nitrogen fed was 17 per cent in each case, a clear indication that the child was utilizing the protein of the vegetable mixture as effectively as that of milk.

TABLE 2

AMOUNTS OF MAJOR NUTRIENTS SUPPLIED BY INCAP VEGETABLE MIXTURE NO. 8 COMPARED WITH ESTIMATED REQUIREMENTS*

	Calories	Protein, gm.	Vitamin A, mg.	Thiamine, mg.	Riboflavin, mg.	Niacin, mg.	Iron, mg.	Calcium, gm.
Recommended*	1140	40†	1.20	0.6	1.0	5.7	7	1.0
150 gm. Veg. Mix. No. 8	750	38	1.17‡	2.1	0.9	12.6	60	1.3

* INCAP's recommendations for children 1 to 3 years old.[46]

† This figure is now believed to be more than double the amount required when the protein is of high biological value.[43]

‡ Vitamin A activity calculated from carotene content.

Amino Acid Supplementation

Now that the industrial production of certain of the essential amino acids at low cost is practicable, the possibility of improving the utilization of vegetable proteins by their supplementation with synthetic amino acids has been discussed.[47, 48] In theory such a measure could be of great value, both for the prevention of kwashiorkor and for the improvement of diets that are protein deficient because of the poor quality of their protein. In most underdeveloped areas in which protein deficiency is a problem, the amount of protein consumed by school children and adults would be satisfactory if only its biological value were higher. In some populations of the Guatemalan highlands the diet contains as much as 75 gm. of protein primarily from corn and still is deficient in tryptophan and methionine; there is also a marked imbalance among the essential amino acids. Thus, amino acid supplementation, if proved safe and effective, should be considered seriously.

Unfortunately, there is not yet sufficient knowledge, either of the availability of amino acids in most vegetable foods or of the optimum proportions for human nutrition, to be sure of the right amounts to add. The work of Elvehjem[49] has clearly shown that small excesses of the essential amino acids have a harmful rather than beneficial effect on protein utilization, and we have recently found that a small excess of methionine added to lime-treated corn consumed as tortillas decreases rather than increases nitrogen retention.[40] It is obvious that much more study is required before amino acid supplementation can be recommended as a practical preventive measure.

Nutrition Education for Prevention

Although economic factors and the unavailability of protein-rich foods are the basic reasons for the occurrence of kwashiorkor, ignorance and food prejudices also play an important role. For this reason nutrition education constitutes a vital part of any program for its prevention.

Central American parents are slow to give supplementary foods to children during lactation, and breast milk may be the only food received by the child until it is weaned. Prolonged lactation is, of course, very advantageous under the conditions prevailing in most regions where kwashiorkor is common, but supplementary food must be given well before the end of the first year. The fear of supplementary foods appears to be based largely on the frequency with which they are associated with diarrhea in young children; such diarrhea is always attributed to the food itself and not to poor hygiene.

At the time of weaning, these prejudices persist or are even accentuated; instead of giving the weaned infant special foods, a selection is made among those received by the rest of the family, which tends to eliminate foods richest in protein. For example, black beans, which are customarily consumed by the adults and which are a relatively good source of protein, are not given to the young child, but he receives instead some of the water in which the beans have been cooked. Even when meat is available to the family, it is not considered a suitable food for the young child, who is given instead only the broth.

An even worse situation occurs when the food intake of the child is drastically reduced because of diarrhea. In this event, solid foods and milk, if the child is receiving any, are withdrawn, and thin cereal gruels (*atoles*) or preparations of starch are given instead. In some of the countries the use of barley or rice or sugar water is customary, and a severe nutritional deficiency rapidly develops. These poor feeding practices for children with diarrhea are found not only at all social and economic levels, but are even encouraged by some physicians. A purgative is commonly given to children with diarrhea on the mistaken theory that it will help get rid of the worms that are believed to be responsible. All of these factors can be combated by nutrition education.

An excellent example of this has been the success of the instructions given to the mothers who come to take their children home after hospital treatment for kwashiorkor. The majority of these children remain in good nutritional condition, as noted on subsequent follow-up visits, even though there have been no obvious changes in the socioeconomic conditions of the family.

Environmental Sanitation for Disease Control

Infectious episodes are frequent precursors of the development of kwashiorkor and have been identified as precipitating or contributing causes. In this connection, special mention should again be made of infectious diarrhea, both because of its primary effect on nitrogen absorption and retention and because of the errors that are made in its treatment. Recent INCAP studies carried out in 2 towns in the Guatemalan highlands showed that an average of 5.5 per cent of children under 5 years of age have clinical diarrhea at any given time. Since the incidence of *Shigella* in these populations varies from 1.5 to 15.5 per cent,[8] it is probable that shigellosis is responsible for a significant proportion of these cases. Amebiasis, malaria, measles, whooping cough, and various other communicable diseases have a similar effect.

It is clear, therefore, that efforts directed toward improving environmental sanitation and controlling communicable diseases should considerably reduce the number of children developing kwashiorkor, even though they will not eliminate the chronic malnutrition present in most of the children in rural and poor urban groups in the so-called underdeveloped areas.

Summary

Severe protein malnutrition occurs in any combination of protein and calorie deficiency, ranging from the adequate caloric intake of classic kwashiorkor to the severe deprivation of both protein and calories that results in marasmus. Furthermore, under actual field conditions, protein malnutrition occurs combined with the deficiency of various other nutrients. Rational dietary treatment must take these variables into consideration, together with the biochemical and pathological alterations that occur in a child with kwashiorkor. Early correction of the electrolyte imbalance accentuated by diarrhea is imperative, but the generally satisfactory nature of the response to dietary therapy alone makes unnecessary the resort to blood or plasma transfusions, intravenous or oral protein hydrolyzates, enzyme preparations, and the therapeutic use of vitamins. On the other hand, the frequency of infection and the lowered resistance of the child make desirable the almost routine use of antibiotics. Intestinal parasites, although frequently present, should not be treated until after the child has recovered.

While the use of milk continues to be an ideal basis for therapy and prevention, properly constituted vegetable protein mixtures are almost equally effective for treatment and are the most promising means of prevention for many

areas of the world. Much more work is required to determine the possible value and practicability of the amino acid supplementation of vegetable foods in areas in which animal protein is in short supply.

Since diarrheal disease is widely prevalent in areas in which kwashiorkor is endemic and contributes to the development of severe protein malnutrition, improved environmental sanitation should reduce the number of cases of kwashiorkor. A major factor in the effective prevention of kwashiorkor will always be nutrition education directed toward improved feeding practices for the weaned infant and the young child.

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PREVENTION AND TREATMENT OF CHRONIC SEVERE INFANTILE MALNUTRITION (KWASHIORKOR)*

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Introduction

Variously known as Shibi Gachaki (Japan), Mehlnährschaden (Germany), Kwashiorkor (Ghana), Síndrome Pluricarencial Infantil (Central America), and Protein Malnutrition (World Health Organization),[1] chronic severe malnutrition is the inheritance of children living chiefly in technically underdeveloped countries. A number of factors social, economic, sanitary, and educational contribute to its high incidence. The cumulative effect of the negative action of each of these factors bears on millions of individuals throughout the world, and the prevention and treatment of the disease have thus become a world responsibility.

Judged at the individual level, this process is the result of the interaction of a poor diet eaten in amounts that fall well below the minimal requirements.

A brief description of the clinical record of a severely malnourished child may be helpful in trying to support some of our ideas in regard to prevention and treatment. A typical dietary history reads as follows:

The mother's diet during pregnancy is in no way improved over her usual one. As a result, the "healthy" newborn is shorter and lighter than normal.[2, 3] For the first 3 or 4 months, breast feeding is more or less adequate, as judged by the weight and height increments of the infant. Thereafter, although the average contents of protein and fat are not too different whether the milk comes from a malnourished or a normal mother, the quantity of milk diminishes in the former.[3, 4] Even though it is true that children continue to suck at the breast until they are 18 to 36 months old, studies by Platt[5] have shown that, at least in Gambia, the average amount obtained is only about one ounce per hour, so that "breast starvation"[6] would be a better term for this than breast feeding. Weaning is instituted abruptly, generally because of a new pregnancy. The child is then fed gruels made with staples such as corn, rice, or millet, and sweetened with cane sugar. The baby is soon affected with bouts of diarrhea, primarily attributed to poor sanitary conditions. These attacks gradually increase in severity; contaminated food is the vehicle for these infections. During the attacks of diarrhea, the dietary intake becomes even more deficient since, as a rule, only corn-meal porridge or rice in water, with added salt or cane sugar, is given to the child. The poor sanitary conditions, which have persisted for generations, are partially responsible for the family's fear of giving other foodstuffs, such as milk, in many communities.

Diet surveys conducted in Mexico, Jamaica, Central America, Chile, Ceylon, and several parts of Africa have shown that the diets consumed by children

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in all of these places have a striking similarity, not only in their nutritional value, but also in the foodstuffs actually eaten: protein foodstuffs of animal origin are practically never ingested.

Considering that the latter foodstuffs, besides being rich in protein, are among those containing the largest amount of fat, it may be said that we are dealing with a diet that is poor in calories, fats, and proteins, high in carbohydrates and containing insufficient amounts of vitamins and minerals. An estimate of the dietary value in 733 children admitted into our hospital because of chronic severe malnutrition gave the data, calculated as percentages of the minimal requirements for their age, set out in TABLE 1.

Regardless of the magnitude of the calorie intake, the ratio of nonprotein to protein calories is always very high.

The infant's diet after weaning is not very different from that of the adults, but reports from Brock[7] in Africa and our own observations in Mexico agree that it is the recently weaned infant who receives the least food. As mentioned, due to the traditional association of food intake with ensuing diarrhea, his diet consists, as a rule, of the watered-down local form of carbohydrate, given in increasing quantities as he grows older.

TABLE 1

MEAN RELATIVE AMOUNTS OF DIETARY ESSENTIALS INGESTED BY MEXICAN MALNOURISHED CHILDREN

Calories	40–50%	Iron	50–80%
Protein	20–60%	Vitamin A	Low
Fat	10–30%	Thiamine	Apparently sufficient
Carbohydrate	60–85%	Riboflavin	Low
Calcium	10–40%	Nicotinic acid	Low
Phosphorus	20–50%	Ascorbic acid	Low

As mentioned before, the chief sources of nutrients in our case are corn (*Zea mais*) and beans (*Phaseolus vulgaris*); wheat is used in small amounts. The amino acid contents of these foods have been determined by Cravioto, Massieu, and Guzmán.[8] When corn meal and beans are mixed in proportions of 4:5, as they are when commonly eaten by Mexican adults, the amino acid content of the mixture is surprisingly good. TABLE 2 shows the corresponding values found in our laboratory as compared to those present in similar amounts of cow's milk protein and to the amino acid requirements of growing infants as given by Holt.[9]

From TABLE 2 it may be seen that except as regards lysine corn and bean protein, when combined in the proportions cited, yield amino acids in amounts equal to or greater than those provided by a similar weight of milk protein. When this mixture was given to rats as their only source of protein, the weight gain per gram of protein consumed was only 0.93 ± 0.037, as compared with a gain of 1.84 ± 0.04 when 22 per cent of the total protein was provided by milk protein.[8]

Since these foods are almost the only ones available for a great majority of the poor population in Mexico, we considered it important to determine the absorption and retention of nitrogen by our malnourished patients, especially since previous observations have shown that some children can be started on their cure when fed large amounts of corn and beans as their only diet.

FIGURES 1 and 2 present the results obtained when nitrogen intake was correlated with nitrogen absorption or nitrogen retention on a diet of corn and beans, as compared to values obtained on a similar group of patients fed cow's milk. Graphs show that both absorption and retention from corn meal and beans, besides being quite variable from child to child, are low as compared with the absorption and retention from milk.

From this it may be concluded that the only food available for the actually or potentially malnourished population of Mexico is poor in quality and quantity.

TABLE 2
MEAN DAILY INTAKE OF ESSENTIAL AMINO ACIDS
(Protein intake—36.7 gm.; body weight—10 kg.)

	Beans + corn	Milk	Requirements
Tryptophan	0.36	0.37	0.30
Valine	2.55	1.74	
Threonine	1.91	1.20	0.60
Methionine	0.64	0.46	0.65
Lysine	1.89	2.35	0.96
Histidine	1.06	0.92	
Leucine	5.87	2.85	
Isoleucine	3.63	1.56	0.90
Phenylalanine	2.69	1.10	0.90
Arginine	1.77	0.83	

Whole milk: 1224 ml.
Bean purée: 360 gm. = 98.3 gm. raw beans.
Corn meal: 240 gm.

Prevention

The problem of the prevention of malnutrition is twofold: better food must be provided, and it must be eaten. In other words, "We not only have to take the horse to the water, but we also must make him drink it."[10]

Space does not permit discussion of methods for increasing food production or for educating people to consume better foods, but these three points should be stressed:

(1) The provision of water and power to open new lands for cultivation is a requirement so universally accepted as to deserve no further comment.

(2) The elimination of pests and crop diseases should receive full consideration.

(3) Methods of cultivation must be changed gradually from the very primitive ones still in use in many communities to fully mechanized agriculture. For example only partial improvement in cultivation technique has more than quadrupled the yield of groundnuts in an African village.[11]

Changes in types of food produced are very difficult to accomplish in areas where the agricultural economy has been fixed by tradition and where individuals possessing very limited holdings of land, after much trial and error, have come close to what they believe to be the most reasonable utilization of their soil.

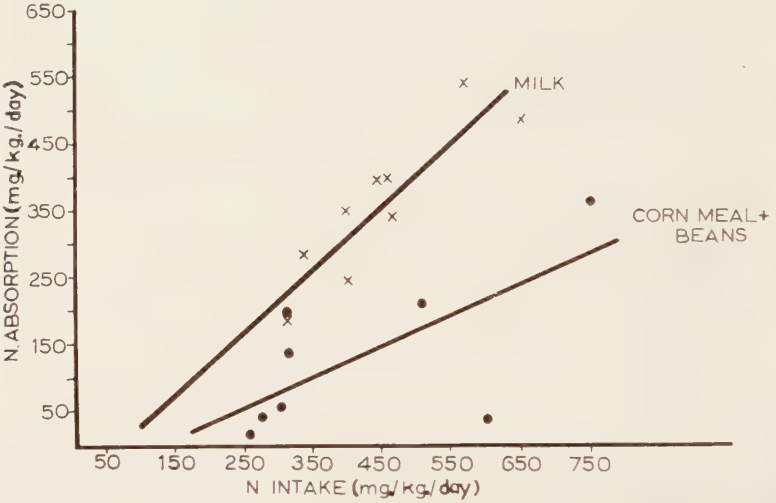

Figure 1. Correlation between nitrogen intake and nitrogen absorption in chronic severe malnutrition.

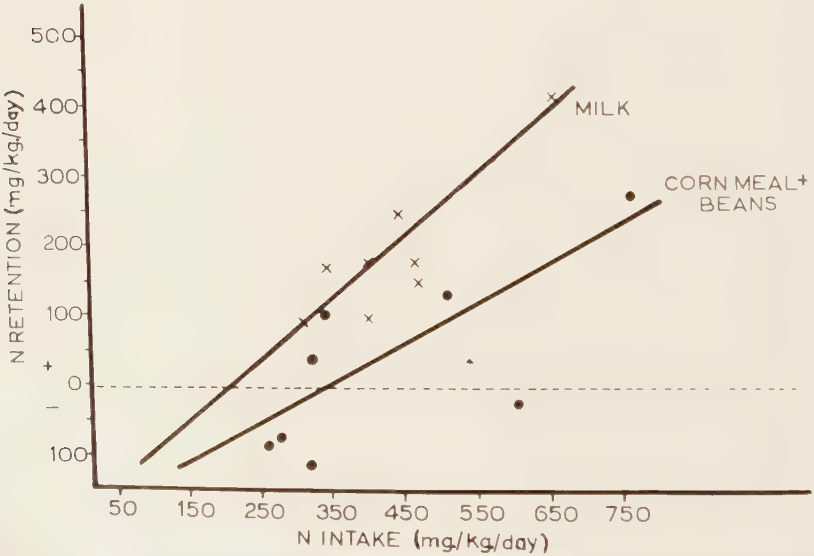

Figure 2. Correlation between nitrogen intake and nitrogen retention in chronic severe malnutrition.

If one cannot influence the type of crop cultivated, the introduction of genetic varieties of higher and better nutrient content has already given good results with corn.

Since the main limiting factors determining the amount of food available to a community are production, transportation, and storage, it may be questioned whether it is wise to increase production of food if it is of low biological value. The result of such a policy might mean only larger amounts of poor food. However, the problem has more important implications if one considers that, in order to get more work done, a better calorie intake is necessary. It has been stated that people living in technically underdeveloped countries are apparently lazy. However, if their physical efficiency is calculated in relation to their caloric intake, it is evident that these people could not possibly survive if more work were demanded from them. If more food is made available to a community, whatever its biological value, this may enable the performance of more work, thus breaking the vicious chain of inadequate food supply→low energy intake→limitation of physical effort→inadequate food supply, ad infinitum. After more food has become available, the next step may be to try to provide proteins of high biological value.[11]

Unfortunately, as regards infants and children, the problem must be viewed from a different angle. Because of the growth demands of the young human, a fully balanced diet is required without any delay or interruption. Protein foodstuffs of animal origin are here the limiting factor, since, as already stated, aside from supplying good proteins, they are rich in fats and therefore in calories. If animal foodstuffs are wanting, a substitute must be found. The general tendency has been to find a local food of high biological value to supplement the deficient diet. However, as emphasized repeatedly, it is practically impossible to change feeding habits once they are established; the supplementary food should therefore meet at least three requirements:

(1) Supplementary food must be pleasing, not only to the child, but to all members of the family as well. It has been pointed out before that the diets of the children are not different from those of the adults, which means that food rejected by the parents is of no avail to the child. It does not matter how perfect a food may be from the nutritional standpoint if it is not going to be accepted. The paramount importance of this point has been insisted upon by Dean,[12] who holds that it is worthwhile to spare no efforts to make sure the food is pleasing, before going further and perhaps setting up a large processing plant.

(2) Once accepted, the supplementary food must be cheap, so that it may be accessible.

(3) The supplementary food must be a rich source of animal protein.

Chemical analyses of local foods and their by-products are accordingly necessary. If the possibility of the use of local foods is indicated by such analyses, a complete assay must be conducted. György has recently summarized the minimal conditions for such an assay: "Any new preparation destined for human consumption should be pretested in infants and older children and in adults. Assays on animals should precede those in human beings. The assays should include study of 'shelf-life,' spoilage on storage, effects of temperature and humidity, etc."[13]

With these ideas in mind, we have set up field studies, selecting 2 comparable villages and conducting a complete survey in them in order to have a base line as regards the economic, social, educational, sanitary, and nutritional status of both communities. Later, one of the villages is to be provided with a supplement of fish meal and, after 6, 12, and 24 months, new surveys will tell us about the acceptance or refusal of this supplement, the causes in either case, and the eventual benefit obtained from it.

Similar studies are being made in other countries. Foodstuffs that have been or are being tried in various parts of the world are soybeans, Brazil nuts, sunflower seeds, peanuts, cottonseed, and linseed. Partial results seem encouraging, and further reports are awaited eagerly.

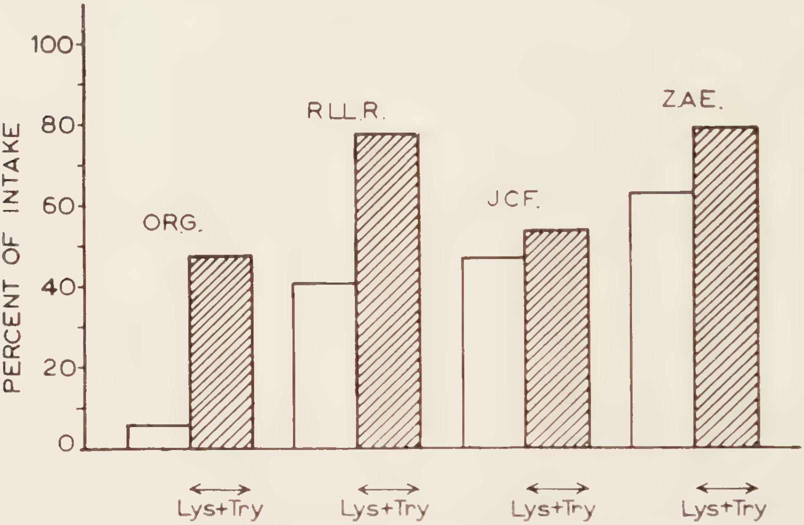

FIGURE 3. The influence of amino acid supplements on the absorption of nitrogen from a diet of corn meal and beans.

Another way of improving the nutritional value of a deficient diet is to supplement it, not with other foodstuffs, but with those special nutrients that are considered to be limiting factors. So far, it has been shown that vitamin B_{12} does not appreciably influence the growth rate of children when used as a supplement.[14] In severe cases of infantile malnutrition we could not observe any significant influence on the rate of recovery when the children were at the same time receiving a fully balanced diet.

The addition of limiting amino acids has given good results in animals. Thus encouraged, we tested a diet of corn meal and beans in which chemical analyses showed that lysine and, possibly, tryptophan were the limiting amino acids. Results obtained by the addition of these acids to this diet are shown in FIGURES 3 and 4. As shown, addition of the supplement in 4 cases was followed by an increased percentage of nitrogen absorption and retention. These preliminary results, presented with the object of demonstrating a potentiality, have encouraged us to proceed into a more detailed investigation.

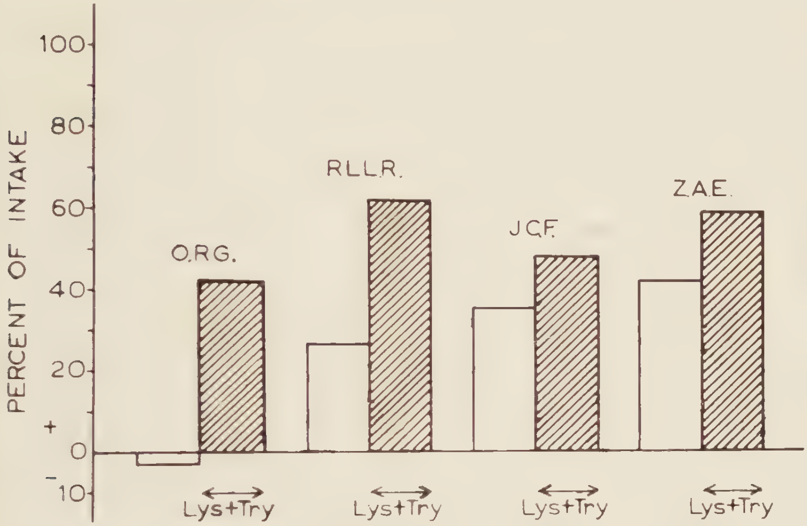

FIGURE 4. The influence of amino acid supplements on the retention of nitrogen from a diet of corn meal and beans.

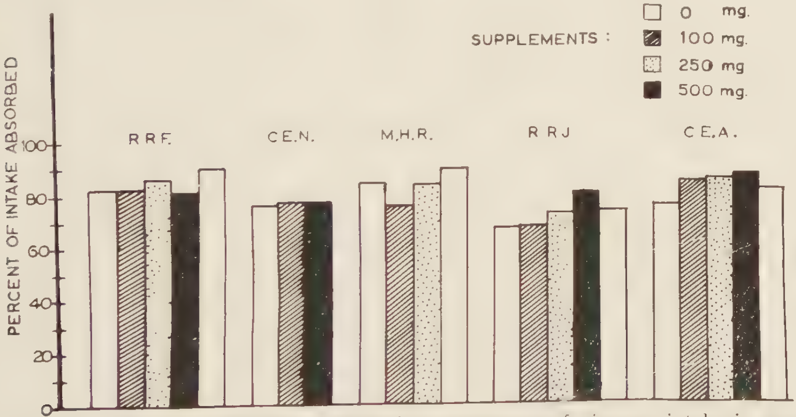

FIGURE 5. Nitrogen absorption expressed as percentage of nitrogen intake in severely malnourished children on a diet of cow's milk with and without L-lysine supplements.

Once a good food is available, the problem of using it becomes one of education. How can we make people eat these foods or supplements? Our idea has been to encourage the finding of items that can be mixed with the normal diet without affecting its original taste since, as previously mentioned, it is practically impossible to change eating habits. Thus, we have been able to wean children on a special mixture, with the consequence that by the time our supplies of it became exhausted the children refused to accept cow's milk. The question of education toward a new food or a supplement is, of course, only a small part of any program tending toward prevention of malnutrition. Trowell[15] and Dean[16] have covered this aspect of the problem in an excellent manner. Three of their points may be emphasized here:

(1) In areas where malnutrition is prevalent, everyone should learn to recognize the disease, especially all adults caring for children.

(2) Recognition of malnutrition at an early stage is obviously a matter of extreme importance. Since there is no method that really can assess the state of nutrition, research in this direction should be given priority.

(3) Mothers and educators should be specially taught the very particular needs of children, and emphasis should be placed on the fact that children must be given absolute priority on food consumption.

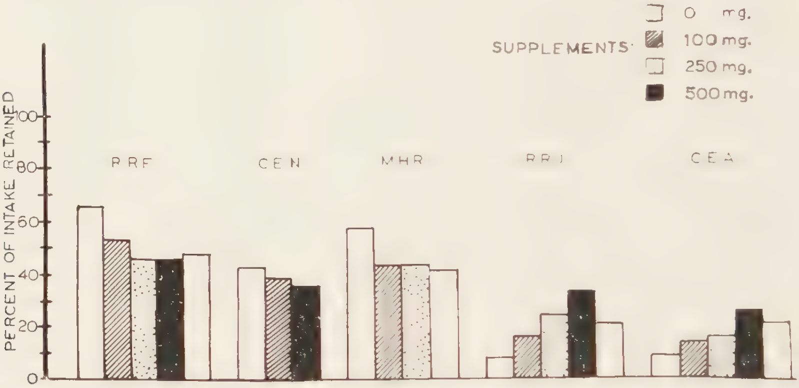

FIGURE 6. Nitrogen retention expressed as percentage of nitrogen intake in severely malnourished children on a diet of cow's milk, with and without L-lysine supplements.

If special care is taken in improving personal and familial sanitary conditions, there is a large group, at least in Mexico, that will be benefited. A demonstration area, both experimental and educational, seems indispensable wherever malnutrition prevails.

One should not forget that diet is not the only factor in human malnutrition. Infections, mainly gastrointestinal, due to poor sanitary conditions and faulty dietary habits and prejudices, may be more important in some places than diet itself. Thus, in chronic severe malnutrition, as well as in any other condition in which many different factors interact, an evaluation of all factors involved should be made first, in order to identify those limiting nutrition, so that a preventive campaign may be planned. In the last analysis, the limiting factors in many places of the world may be of a cultural and socioeconomic nature rather than specifically dietary.

Treatment

In spite of increasing knowledge as regards preventive measures, infantile chronic severe malnutrition is still a great scourge among children in the whole world, mortality varying between 20 and 60 per cent in the majority of technically underdeveloped countries. Prognosis has proved to be dependent chiefly upon severity of malnutrition as judged from weight deficit and upon certain complicating features of the disease, especially those of an infectious or metabolic nature. Mortality due to water and electrolyte disturbances related to acute diarrheal episodes or to respiratory infections, chiefly bronchopneumonia, is greater during the first 48-hours spent by patients in a hospital, and its incidence is generally not affected by the use of recommended therapeutic measures.[17] According to the presence or absense of acute complications, treatment of chronic severe malnutrition will conform to different therapies. In the first case, management obviously comprises 2 chief steps: (1) an attempt to save the life of the child during the most critical early period of hospitalization, and (2) if this is successful, initiation of the necessary measures tending to restore nutritional equilibrium.

The first of these steps, which is properly an acute emergency and not the treatment of malnutrition per se, is mentioned here because of the frequent occurrence of failure in attempting it — a result probably related to an inadequate knowledge of homeostatic mechanisms in malnourished organisms. In a child who has suffered from malnutrition since the early days of his life, evaluation and treatment cannot possibly be made according to concepts derived from observations made on well-nourished organisms. An accurate knowledge of features of the complications of malnutrition, as in the particular case of water and mineral metabolism, may eventually be obtained from study of tissue composition and renal function.

From the clinical standpoint, water and electrolyte disturbances in a malnourished child are commonly characterized by these five symptoms: (1) apathy; (2) lack of thirst; (3) polyuria, hyposthenuria, and isosthenuria; (4) coexistence of clinical features of dehydration and edema; and (5) lack of usual signs of systemic or local infection.

Biochemical investigations in these same subjects have shown: (1) relative increase of extracellular fluid volume; (2) extracellular hypotonicity; (3) intracellular overhydration; (4) decrease of intracellular potassium concentration, not necessarily revealed by hypokalemia; and (5) intracellular water and sodium shifts upon decline of patient.

Studies of renal function have demonstrated: (1) decrease in glomerular filtration rate and renal plasma flow; (2) high clearance of osmotically free water; and (3) decreased sodium and phosphate excretion.

Treatment of the acutely ill child, suffering primarily from chronic severe malnutrition, necessarily must take into account many of the clinical and biochemical paradoxes apparent from this summary. Thus, the use of a hypotonic solution would probably be contraindicated in an already hypotonic subject. On the other hand, while treatment with hypertonic solutions would seem logical, their use may be potentially dangerous because of uncontrollable water shifts within the organism. At present, therefore, the most adequate repair solution seems to be one having a similar osmolarity with the patient's own blood plasma and supplying the necessary potassium and calcium ions. At any rate, the extremely delicate balance upon which survival of an undernourished dehydrated child depends precludes the recommendation of a universal good-for-everything solution. Perhaps more than anywhere else in medicine, these individual cases require individual attention. The same may be true in the treatment of infection per se, if occurring in a malnourished subject.

Treatment of malnutrition is etiologic: it consists of supplying food. Our group has summarized the treatment of chronic malnutrition by saying that it consists of a "normal diet, to be consumed freely, rich in calories and proteins of high biologic value, palatable, of easy ingestion and digestion."[18] Vitamin supplements have thus far been found unnecessary.[19]

Thus, children who have survived the infectious accident or the acute electrolyte disturbance, or those who have been spared these situations, are given initially a liquid diet, preferably whole milk with 10 per cent corn syrup added. Ingested ad libitum, such a diet does not provide, at first, more than 35 cal./kg. of actual weight, or 20 cal./kg. of ideal weight. Nevertheless, such a low calorie and protein intake is sufficient, during the first week, to achieve a positive balance. This fact must be taken into account in order to avoid the common error of branding such a child as "anorexic." Gradually but quickly, the patient is capable of ingesting larger amounts of food and, within 15 days, he is already receiving 100 or more calories per kilogram of actual body weight. By this stage, the intake of foods other than milk, mainly puréed meat and vegetables, already has begun.

TABLE 3

AVERAGE VALUES PER KILOGRAM OF BODY WEIGHT OF NUTRIENTS IN DIETS INGESTED ON SELF DEMAND BY 373 SEVERELY MALNOURISHED CHILDREN

	First 5 days	6th to 10th day	11th to 15th day	Thereafter
Calories	35.0	61.0	110.0	190.0
Carbohydrates (gm.)	6.5	11.0	17.0	26.0
Proteins (gm.)	1.3	2.0	3.0	6.7
Fats (gm.)	0.4	1.0	3.3	6.4
Vitamin A (I.U.)	200.0	300.0	700.0	7300.0
Ascorbic acid (mg.)	3.5	5.0	15.0	120.0
Riboflavin (mg.)	0.7	1.0	1.7	3.0
Niacinamide (mg.)	0.5	0.5	0.6	4.5
Thiamine (mg.)	0.1	0.2	0.3	0.8

A small number of children show actual anorexia, which is extremely difficult to fight. In such cases, feeding by a slow and continuous gavage through a polyethylene tube has been successfully accomplished. Only rarely has it become necessary to employ this resource for more than 5 days.

Three weeks after admission, under such a liberal "self-demand" regimen, the patient is ingesting 200 to 250 cal./kg. of body weight through a varied diet. From the foods ingested by a group of 373 children, we calculated the following average values of the nutrients

Treatment may be considered successful when the patients show:

(1) Quick improvement in appetite and interest in food.

(2) Progressive weight increment. A considerable proportion of children show gains from 50 to 100 gm. daily during recovery; this seems incredible to those not familiar with the disease.

(3) Disappearance of edema. With no therapeutic measure other than diet and rest in bed, it is frequent to see edema, which has been calculated to represent from 10 to 25 per cent of actual body weight, disappear in a matter of 10 to 15 days. Because of the severe depletion of electrolyte shown by these patients, we are afraid to use other agents to attain a more rapid mobilization of edema, as such measures generally produce further depletion of sodium and potassium. In a few cases, edema in these patients becomes worse due to congestive heart failure, apparently also of nutritional origin. Treatment in these cases obviously must include measures intended to prevent deterioration and failure. In these cases, success is not achieved with thiamine.

(4) Clinical cure of skin lesions. Regardless of the treatment employed, "pellagrous" skin will peel off in a matter of 10 to 20 days, leaving a new skin with more or less normal characteristics.

(5) Normalization of serum albumin levels. While total protein concentrations in blood serum will become normal in about 20 ± 5 days, albumin levels are a much more precise index of improvement; they do not turn normal before 45 ± 15 days. This development occurs together with a further increase of gamma-globulins, which must have been already high on admission of the patient to the hospital.

(6) Clear signs of well-being It is a well-known fact that the return of appetite and smiles in a sick subject are signs of good prognosis. Later on, other manifestations of a normal social behavior and of neuromuscular rehabilitation appear.

In a few cases, usually in infected children, anemia is so severe, even if it belongs to the typical normocytic, normochromic type, that measures such as packed red cell transfusion seem indicated. However, according to our experience, even totals of 3 million red cells per cu. mm. or 7 gm. hemoglobin per cent do not require transfusions. Rather, an adequate diagnosis and early treatment of an existing infection seem to be more important. In this type of patient, even the presense of megaloblastic anemia does not generally establish indication for any treatment other than dietary. Eventually, hypochromic anemias in recovering children may require the use of iron, if one cannot be sure that they will continue receiving an adequate diet at home for a sufficient period of time.

If treatment is conducted with success, the anatomical, functional, and biochemical lesions disappear gradually, following a reverse order from that in which they originally appeared.

Aside from the total or partial regression of lesions due to malnutrition, a series of clinical and biochemical alterations appear during the 2-month period after the beginning of treatment. These symptoms have been described collectively under the name of nutritional recovery syndrome.[20] The chief clinical signs appear 15 to 45 days after treatment has been initiated and last for about one month. They may be observed in all forms of advanced malnutrition and are coincident with the healing of skin and mucosal lesions. They are:

(1) Progressive hepatomegaly, which is characterized histologically by the disappearance of steatosis, if fatty degeneration has previously existed.

(2) Potbelly, with ascites and superficial venous network on abdomen and chest.

(3) Hypertrichosis, predominant on the forehead, cheeks, shoulder, and thighs.

(4) "Moon face," comparable to that of Cushing's syndrome, and telangiectasis.

(5) Progressively increasing hypergammaglobulinemia.

(6) Progressively abnormal thymol turbidity, which parallels the evolution of gamma-globulin levels.

(7) Increase in blood volume, with simultaneous transitory decrease of hematocrit and hemoglobin concentration, followed immediately by reticulocytosis. These findings occur simultaneously with the appearance of potbelly and ascites.

(8) In 33 per cent of the cases, eosinophilia, not related to allergy or parasites.

(9) About 100 days after the beginning of treatment, long bones show a zone of subepiphyseal condensation, clearly delimited from the osteoporotic metaphyses. At the same time, calcium, inorganic phosphate, and alkaline phosphatase concentrations in blood are abnormally high.

These manifestations of the nutritional recovery syndrome may all appear in a single case, or only some of them may be present simultaneously. They have been considered the result of accelerated growth and development in a previously nongrowing subject, with varying increments for each different organ, and their pathogenesis is at present under active study.

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Discussion

Paul Gyorgy (*University of Pennsylvania School of Medicine, Philadelphia, Pa.*): The excellent presentations of the preceding three essayists have delved into a rich store of observations and have acquainted us with the rather remarkable progress achieved during the last few years in the newer knowledge on the pathogenesis, prevention, and treatment of kwashiorkor.

I propose to touch upon a few points only. First, the "pyramid" of Béhar and Scrimshaw is an extremely helpful concept. It should be emphasized, however, that marasmus may develop without the intermediate stage of prekwashiorkor or kwashiorkor. In total caloric starvation or undernutrition with a food such as milk, we have no prekwashiorkor. We have prekwashiorkor or kwashiorkor only when the ratio of calories to protein is really disturbed. I refer not only to the quantity of protein, but also to the quality of protein, which is usually a factor.

The calorie-protein ratio has been mentioned once in the papers under discussion. Its formulation is an extremely difficult problem. In caloric and protein starvation (I am now speaking of milk protein), there is also protein depletion and no growth. However, we have no fatty liver, no hypoproteinemia, and no reduction in intestinal enzymes. According to the teachings of classic physiology, increase of the nonnitrogenous calories should improve protein retention. In practice, however, we see here the slow and progressive transition to kwashiorkor.

In studies not completed that I have carried out in collaboration with D. D. Kornfeld, rats on a soybean protein diet responded to increase of nonnitrogenous calories with improved nitrogen retention but, at the same time, they developed fatty liver, then cirrhosis; as if the nitrogen (in accordance with our contention) did not go to the liver, the nitrogen content of which was found to be unchanged, but to other tissues, probably to the muscle. We do not know where the break occurs, but it must occur.

Then we have what we call, as I still prefer to name it, kwashiorkor or prekwashiorkor— an entity that I like to maintain.

As regards treatment, I have been very much impressed by the excellent results that Jean Sénécal has obtained with protein hydrolyzate: milk protein-hydrolyzate, as I understand it. However, these results still remind me of the state of affairs a few years ago in the treatment of liver disease.

Dietary liver disease is considered a manifestation of protein deficiency, and my associates and I have recommended for its treatment diets high in protein. However, we have often encountered difficulty, especially in severe acute hepatitis or in decompensated liver. Protein hydrolyzates, 9 to 12 gm./kg. from the start, may lead to trouble. I feel it necessary to utter a word of warning, especially when I see the tables and the results of Béhar and Gómez, who proceed very, very cautiously and slowly. These workers gave only 1.3 gm./kg. at first, increasing this amount progressively to 2, 3 and, later, more, and they achieved good results. Then we come to an interesting new concept, a really new discovery made by Gómez; namely, the recovery syndrome. This is a new concept. I have never heard of it before. It has not been described previously insofar as I know, and it is an amazing syndrome. However, I think that we are dealing here with the same set of symptoms that we observed in our war prisoners who returned from Japan and developed similar endocrine troubles after refeeding. I think there the refeeding was very fast. These men were very hungry, they ate fast, and they developed these endocrine disturbances.

I should like to prevent the recovery syndrome. I should like to teach the children not to eat too much food; to be a little more satisfied with less.

With regard to treatment and prevention of kwashiorkor, I think the results, especially of INCAP, are extremely impressive and, having had the privilege of watching them more or less since the start of these studies, I congratulate Scrimshaw, Béhar, and their group on the excellent results they obtained.

Finally, a word of thanks to Gómez, who comes from Mexico, a country of very old and admirable culture. In a very diplomatic and tactful manner he has spoken of *technically* underdeveloped countries. I am grateful to him for teaching us that we should not speak simply of underdeveloped countries. We should speak, instead, of "technically underdeveloped countries."

H. E. SAUBERLICH (*Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.*): My comments are not concerned directly with kwashiorkor as observed in humans, but rather with an experimental condition, produced in rats, that resembles the disease. I refer to the condition as experimental nutritional edema. This edema is produced readily by feeding rats diets low in protein, methionine, choline, folacin, and vitamin B_{12}.[1, 2] Generalized edema with a 100 per cent incidence can be produced in rats in 10 to 12 weeks. The liver of the edematous rat is fatty and cirrhotic. The degree of cirrhosis is variable, since it is related to the diet used, the age of the animals, and the time of onset of the edema. Damage to the pancreas and the heart is usually noted. Addition to the diet of protein, choline, methionine, betaine, or folacin and vitamin B_{12} will prevent the development of edema.

Analyses of the blood and liver demonstrate changes similar to those reported for kwashiorkor. Enzyme and nucleic acid concentrations of the liver are altered markedly. The blood pressure of animals on the edema-inducing diets falls and, at the onset of edema, may fall to very low levels. The serum electrophoresis patterns of the edematous rats are quite different from those of the control animals. The alpha$_1$-globulin fraction is reduced or absent in the edematous rat. The beta- and gamma-globulins often increase, particularly when severe cirrhosis occurs. The albumin falls, but more slowly than does the alpha$_1$-globulin. However, in very acute edema the serum albumin may be reduced to very low levels.

Rats with edema have been treated successfully with diets containing high levels of protein, choline, and vitamin B_{12} and folacin. However, several weeks of feeding were required before the serum and blood pressure returned to normal. Supplements of choline alone required a long period of time for recovery. Even the hemoglobin level returned to normal slowly.

Although the supplements of choline, methionine, and folacin and vitamin B_{12} prevent the occurrence of edema, completely normal livers are not observed; for instance, fatty livers occur. To prevent this infiltration of fat, other amino acids or higher levels of complete proteins must be administered. Partial deficiencies of amino acids other than methionine have not produced edema routinely.

The experimental edema appears to be associated with methionine and methyl groups, as in choline or, indirectly, in vitamin B_{12} and folacin. The condition may be associated, in turn, with impaired liver function or insufficient methionine for the formation of plasma proteins. Secondarily, dietary protein and other amino acids would be associated with the condition.

There appears to be a similarity in the changes that occur in the animal with experimental edema and those seen in the human patient with kwashiorkor. Thus, it would be of interest to know if the condition that occurs in humans could be prevented by dietary supplements of methionine, choline, and folacin and vitamin B_{12}. Supplements of lysine, tryptophan, and threonine also might be included, since these amino acids have some protective effect in the edema studies and aid in the prevention of the infiltration of fat into the liver.

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JACK METCOFF (*Michael Reese Hospital, Chicago, Ill.*): I propose to comment on two or three items mentioned in the very interesting and excellent papers presented thus far and then to amplify some of the observations reported by Gómez. It was my privilege to have the opportunity of working with him and his group a few years ago.

Lysine is one of the amino acids considered important in relation to the development of the syndrome of kwashiorkor. It is of interest that, in potassium-deficient rats, lysine appears to be one of the amino acids that is thought to substitute for potassium in the cells, or at least to contribute cationic equivalence, since lysine is a divalent cationic amino acid. This seems somewhat incompatible with the concept of simultaneous potassium and lysine deficiency. Similarly, a number of years ago it was thought that protein deficiency produced deficiency in gamma-globulin and, hence, a reduced resistance to infection. Children with kwashiorkor tolerate infection poorly, have many infections, and yet tend to have hypergammaglobulinemia. Another point that seems of interest, as my colleagues who deal with protein malnutrition have pointed out to me, is that in some areas the incidence of the edematous phase is partially seasonal. In those countries where there is a rainy season, there is a greater preponderance of the edematous form of the disease at that time. The dry season tends to be associated with the less edematous form of the disease. It is conceivable that foods available during the rainy intervals differ from those eaten in the dry season.

I should like to describe some features of this syndrome that were observed during the first 48 hours of hospitalization. My colleagues and I were concerned with this period because in many instances death was attributable to extensive derangement of the fluid balance. This derangement did not respond to the well-known and effectively used types of electrolyte therapy. It seemed of interest, therefore, to explore the nature of this process.

Characteristically, the muscle composition of children with profound malnutrition was found to be of two general types; the malnourished children tended to have either an increased extracellular phase or a decreased extracellular phase in muscle. However, whether these patients had an increased or a decreased extracellular phase, an increase in intercellular phase was noted. The hypotonicity noted by Gómez seems to be very striking in the intracellular phase as far as potassium and sodium concentrations, at least, are concerned. The discrepancy between the sum of Na + K, making allowance for magnesium, suggests that an intracellular defect of phosphorus also might exist. The relationship of potassium to noncollagenous nitrogen was altered in the malnourished children irrespective of whether they had dehydration or edema. The reduction in K/N ratio was not due to a reduction in the potassium content of the muscle; it appeared to be due to an increase in the noncollagenous nitrogen content of the muscle. This probably represents a "cannibalizing" of the material derived from muscle masses that had disappeared previously. From a child who ultimately died we had an opportunity to obtain two muscle biopsy samples; one at the time of admission, 5 days before death and, by chance, another one 32 hours before death. The characteristic feature appeared to be a shift in muscle water with a decrease of extracellular water and an increase of intracellular water. There was a markedly increased concentration of sodium within the cell that was not associated with any change of potassium content in the cell; that is, an accumulation of water and sodium without an associated deficit in potassium. The opposite phenomenon also was observed. A child was admitted with severe malnutrition; this patient had a contracted extracellular phase and expanded intracellular phase in muscle. With recovery over a 25-day period there was a return toward normal composition of the muscle. A decrease in concentration of sodium within the cell from 33 to 12 mM per liter of intracellular water was observed.

Renal functions in relation to malnutrition were studied principally by G. Gordillo. Malnourished children have marked reduction in renal function in relation to surface area; that is, about one half the normal glomerular filtration rate and renal plasma flow. We had an opportunity to observe one particular child while his condition deteriorated somewhat following an episode of diarrhea and dehydration. This patient showed a striking fall in total renal plasma flow and also in glomerular filtration rate. As Gómez has mentioned, the hypotonicity of these children is associated with an increased excretion of dilute urine even in the presence of severe dehydration.

We have attempted to draw a tentative hypothesis of the development of these biochemical lesions. We visualize the protein and caloric insufficiency leading to physiologic changes, associated with clinical features of poor weight gain or weight loss. Diarrhea or infection lead to changes in extracellular and intracellular fluids that are superimposed on those caused by protein and caloric depletion. Net intake of water exceeds that of solute, causing hypotonicity. With hypotonicity, edema occurs and is associated with initial cellular adjustment in tonicity and increased cell volume. Continued diarrhea causes dehydration; fluid and salt loss is superimposed upon an already increased intracellular water. The renal adjustments allow the child to undergo either compensation or decompensation ("athrepsia"). If decompensation occurs, the child may still have some degree of edema or dehydration, but he will be in a state of limited physiological flexibility. Thus, any event such as infection or diarrhea may cause death rapidly. With compensation, the child becomes marasmic. The marasmic child actually seems quite resistant to sudden electrolyte stresses, possibly because of the reconstitution of surviving cells as a result of effective uptake of endogenously released materials from previously destroyed cells.

Nevin S. Scrimshaw (*Pan American Sanitary Bureau, Regional Office for the Americas of the World Health Organization, and Institute of Nutrition of Central America and Panama* [*INCAP*], *Guatemala, C. A.*): In 1955 the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations sponsored jointly with the Josiah Macy Jr. Foundation of New York, N. Y., a conference on Human Protein Requirements and Their Fulfillment in Practice, held at Princeton, N. J., in which persons working actively with kwashiorkor in ten different countries in three continents were brought together.[1] As the discussions proceeded, it became strikingly apparent that all of these participants were discussing essentially the same syndrome, despite minor differences in the ages at which onset of the disease occurred and the nature of associated secondary deficiencies.[2] In these pages we note the similarity in the approach of workers from three different countries and two continents to the prevention and treatment of kwashiorkor.

Not only is kwashiorkor basically the same clinical syndrome the world over, but also the epidemiological factors are more or less the same. Two features stand out: (1) kwashiorkor occurs most commonly soon after weaning, when the diet substituted for mother's milk is deficient in protein of good quality, and (2) the problem is complicated by stress factors in the environment, particularly the frequency of infectious disease. However, the number of children with kwashiorkor is merely an index of the prevalence of protein malnutrition in almost the entire preschool age population in some areas.

Furthermore, protein malnutrition in this age group is a reflection, not only of poor feeding practices, but also of a chronic shortage of protein-rich foods in the area.

The management-consultant firm of Klein and Saks, Washington, D. C., has recently completed a study in Guatemala in cooperation with INCAP that illustrates the deficit between food production and nutritional needs characteristic of underdeveloped areas in general. In FIGURE 1 the estimated requirement for each food group was based on the minimum amounts necessary for an adequate diet. For purposes of the calculation, as little change as possible, consistent with nutritional adequacy, was made in present urban and rural dietary patterns. Maximum use was made of corn, beans, and other basic foods now consumed in large quantitites, so that the amounts of animal products listed as required fall far short of United States standards. It is highly significant that the only food group available in an adequate amount, even on this minimum basis, was that of refined carbohydrates, principally sugar. Over 160 per cent of the desirable amount of this source of "empty" calories in the diet was produced. Only 68 per cent of the minimum "needed" amount of cereals was produced, however, and less than 50 per cent of all other food groups. Of course, some of the deficit is made up by imports, but these imported foods are frequently not available to the lower-income groups. It is easy to understand why protein deficiency is so widespread.

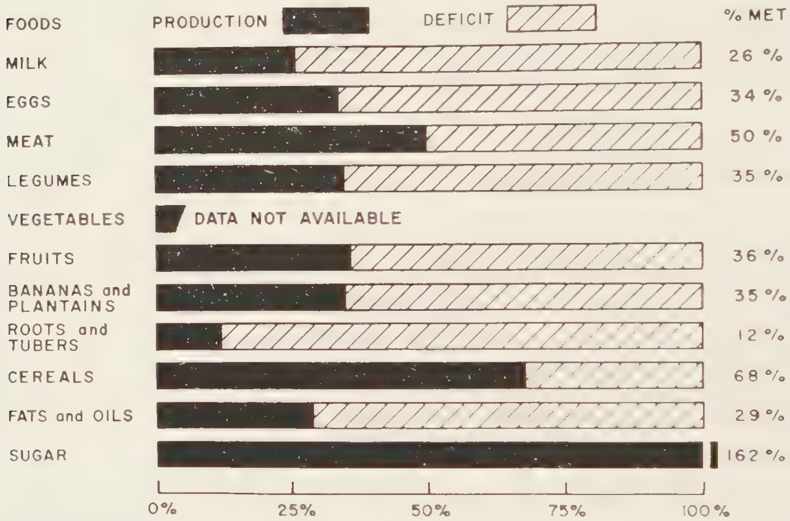

FIGURE 1. Available food production compared with actual food requirements in Guatemala in 1955. (Prepared by Klein and Saks, Washington, D. C., in collaboration with INCAP.)

As has been pointed out, the prevention of kwashiorkor will require major changes in the way young children in underdeveloped areas are fed. Of 22 children investigated by us because of admission to the hospital with kwashiorkor, none had received meat, cheese, or fish, 3 had received an occasional egg, and 9 had received cow's milk, but usually only in token quantities given in a dilute solution.[5] All but one had received the thin cereal gruels known as *atoles*, and most of them had received coffee, but 8 of them had not even been given tortillas and beans, the staple solid food of the adult diets. It is obvious that extensive education will be required, as well as an inexpensive supply of suitable protein-rich food or foods.

These discussions have revealed some degree of confusion regarding the relationship between marasmus and "classic" kwashiorkor, especially since all three of the papers under discussion have dealt primarily with the treatment and prevention of that combination of calorie and protein deficiency referred to as marasmic kwashiorkor. As Béhar showed in a schematic representation of the different types of malnutrition in the form of a pyramid (FIGURE 1 in his paper), certain characteristics are associated with the chronic starvation of marasmus, and others are associated with the protein deficiency in the presence of adequate calories that is responsible for classic kwashiorkor. As seen in most regions of the world, however, kwashiorkor is complicated, to a greater or lesser degree, by a chronic deficiency of calories, and is characterized by a marked tissue wasting and loss of subcutaneous fat not found in the so-called "classic" kwashiorkor. As one moves in the spectrum close to the deficiency of all nutrients that characterizes marasmus, the signs of kwashiorkor become less pronounced.

There is evidence that certain of the biochemical changes that are characteristic of kwashiorkor do not occur in marasmus, and certainly neither fatty liver nor edema occur in the latter. György, in his comments, emphasized the importance of distinguishing between the two conditions, and the pyramid used by Béhar to portray infant malnutrition does this clearly and graphically. However, it is also necessary to recognize that the majority of cases studied and treated by Sénécal, Béhar, and Gómez are neither at one extreme nor the other, but fall somewhere between them.

While the base of the pyramid represents the clinically recognized cases of marasmus, marasmic kwashiorkor, and kwashiorkor, the bulk of it is composed of a continuous range from a well-nourished condition to severe malnutrition. The farther from normal, the more clearly the characteristics of malnutrition become evident, and the more readily it is possible to distinguish between marasmus and kwashiorkor.

Gómez speaks of "third-degree malnutrition," by which he means a weight of 40 per cent or more below the normal weight for well-nourished Mexican children. The pyramid used by Béhar also could be visualized as divided transversely into 3 segments: (1) that nearest the apex to include first degree malnutrition (10 to 25 per cent underweight), (2) the middle, second-degree malnutrition (25 to 40 per cent underweight), and (3) that nearest the base, third-degree malnutrition. This would demonstrate the concept that I believe to be correct; namely, that the term third-degree malnutrition, as used by Gómez, includes marasmus and marasmic kwashiorkor as well as, theoretically at least, any other nutritional cause of severe weight loss. Usually, third-degree malnutrition without edema is marasmus, although it may also include an occasional case of kwashiorkor in which the edema has already disappeared. In Mexico and Central America, third-degree malnutrition with edema is almost always marasmic kwashiorkor. Because little weight loss is involved, classic kwashiorkor ordinarily would not be covered by the term third-degree malnutrition. I believe that this explanation reconciles the terminology of the principal contributors to this portion of this monograph.

Sénécal, Béhar, and Gómez have all emphasized the importance of finding ways of using vegetable as well as animal protein for the prevention of malnutrition, and all have presented data that showed substantial progress in achieving this result. Workers in nutrition in the United States are being increasingly called upon for assignment as foreign consultants. It is important to recognize this and to teach students to seek all possible solutions to the problem of protein malnutrition, including maximum utilization of vegetable protein sources, rather than to expect that North American production and comsumption levels of animal protein can be applied reasonably to all areas.

The evidence presented in these pages on nitrogen balance studies in children to the effect that the nitrogen retention with a suitable vegetable mixture is essentially the same as that with milk has particular significance. This has long been discussed as a theoretical possibility, and it is encouraging to see that the concept of complementary vegetable protein combinations is being applied successfully to the solution of the major problem of improving protein nutrition and, as a corollary, preventing kwashiorkor. The contributors deserve commendation for their practical accomplishments as well as the scientific excellence of their work.

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CERTAIN BIOCHEMICAL FINDINGS IN MAN IN RELATION TO DIET*

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This paper deals primarily with observations made on the South African Bantu, upon whom, with a number of associate workers, I have been carrying out various investigations over the last ten years. The local Bantu differ from the white population in numerous ways: in diet, biochemistry, hematology, physiology, and pathology. In some respects, these people are the objects of our sympathy; in others, they are most certainly the objects of our envy. In some countries there would seem to be a limited choice of problems in human nutrition to investigate, but in South Africa it is a question, rather, of which fascinating problem one should attack first. In the course of my work I have touched upon many fields, any one of which could well engage the attention of several workers for many years. I have endeavored to investigate some problems intensively; I have merely scratched the surface of others. Although the Union of South Africa is about one sixth of the area of the United States, its white population is about one fourth of that of New York, N. Y.; its total population is less than one tenth of that of the United States. Having a Bantu population living on one's doorstep, so to speak, causes one to realize that in South Africa the fields of research are ripe for harvest and that the laborers are far too few.

Diet

The Bantu of South Africa number about 9½ million: accordingly, they greatly outnumber the white population of about 3 million. In addition, there are about 1 million Eurafricans and one half million Asiatics. Of the Bantu, roughly one third dwell in Native Reserve areas, about one third work on farms owned by white people, and the remainder are urbanized, working in government and municipal departments, in industry, and as domestic servants. These people, therefore, may be observed in various stages of dietary transition, from the primitive living off the land to the urban houseboy consuming a largely Europeanized diet. The Bantu diet, speaking generally, includes a large amount of cereals, which supply 50 to 90 per cent of the total calories in the food consumed. Such cereals, usually whole-ground or lightly milled, comprise maize (corn), "kaffir corn" (*Sorghum vulgare*), and wheat. The maize and kaffir corn products are eaten, not only as a variety of porridges, but as fermented foods (such as *marewu*, *lambalaza*, and kaffir beer). These foods, being traditional, are widely consumed, often in large volume. This particular characteristic will be referred to again. The wheat, of course, is consumed as bread (about one half white and one half whole grain bread); its consumption is becoming increasingly popular near and in the large centers of population, perhaps principally because it is a food immediately ready for consumption. A further occasionally important item of diet consists of different types of legumes. Of vegetables consumed, the more common ones, particularly in rural areas, are the pumpkin, sweet potatoes (*Ipomoea batatas*), and various greens. The amount of eggs, meat, fish, and dairy produce eaten, also sugar, is usually much less than among the white population. Briefly and very generally, the diet of these people, according to accepted standards, although usually adequate in calories and gross protein, is low in animal protein and fat, high in carbohydrate and crude fiber, high in certain mineral salts and vitamins, for example, phosphorus, iron, vitamin A, and thiamine, but low in others, for instance calcium, riboflavin, and vitamin D.

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This pattern of diet is common; in fact, qualitatively it is the pattern consumed by the greater part of the earth's population. The same pattern of diet was common among our forefathers a century or so ago; moreover, the adoption of such a regimen frequently is forced upon many countries for limited periods by wartime restrictions.

What are the likely effects of the lifelong consumption of such a diet? What are likely to be the biochemical and pathological stigmata of a deficiency, or perhaps of an excess, of nutrients? Those who have participated in discussions on the examination and revision of recommended allowances will know of the heart searching that arises when we ask ourselves: How much do we really know about human needs of nutrients? Often one reads phrases such as "minimum physiological requirements," "marginal and optimal intakes," and "safety margins"; surely such expressions bear witness to the incompleteness of our present knowledge of the subject. Moreover, those of us who are white and live alongside a nonwhite population such as the South African Bantu must confess to the exasperation we often experience on considering whether these people are or are not short of this or that nutrient, particularly when, as often occurs, the relevant deficiency stigmata are not apparent.

I now cite a few examples of nutritional situations and their ramifications confronting those of us in South Africa who are interested in the broad picture of the diet, in biochemistry, and in the clinical state and pathology of the Bantu. The examples to be described, which bear on protein, fat, calcium, iron, niacin, and vitamin C, in some instances call for a revision of current thought on diet and certain diseases; in others, they indicate a need for the reassessment of recommended allowances of nutrients; while other examples touch on phenomena deemed to be abnormal or even pathological, but which nevertheless would seem to be the normal response of healthy persons to the pattern of diet consumed.

Protein

The first point to be emphasized is that, in contrast to many other African populations, the diet of the South African Bantu older child and adult is characterized neither by deficiency of gross protein nor of specific amino acids.

According to recent local dietary surveys,[1-3] the gross protein intake of the adult South African Bantu is approximately adequate on the 1 gm. kg. basis. Indeed, since the bulk of the energy value of the Bantu diet is derived from cereals (roughly 400 to 600 gm. per diem, depending on the region), and since significant underweight (perhaps 10 to 15 per cent less than weight/height tables indicate) is not a feature of these people, the gross protein intake, in the main, *cannot* be seriously low. This observation must not be taken to imply that *all* local Bantu adults obtain adequate protein. Obviously, among these people, as among any other population group, a proportion ingest insufficient of the nutrient. The fact remains that a low total protein intake is not *characteristic.*

Concerning the intake of amino acids, if one takes the amino acid needs of young American adults reported by Rose[4] and assumes that the figures are applicable to the Bantu pattern of diet, then simple calculation shows that these amounts are contained in about 300 to 350 gm. maize or combination of cereals –an amount contained in the diet of most Bantu. Indeed, when one considers the *total* diet of these people, the diet usually contains more than the remommended allowances of amino acids suggested by Rose, that is, the amounts that were multiplied by two to provide a "safety margin." Patwardhan,[5] from an examination of diets consumed in several different countries, has also concluded that where the calorie needs of a population are met and satisfied largely from cereals, the intakes of the different amino acids are reasonably satisfactory. However, as Patwardhan pointed out, one does not know whether amino acid needs are or are not significantly affected by different patterns of diet. The foregoing does not apply to the child, whose diet, from weaning until he reaches school age, is very likely, especially at the beginning, to be deficient in protein, among other things. Nevertheless, taking into account, first, the level of intake of gross protein of the local Bantu and, second, the increasing evidence strongly suggesting that the protein requirements of man are less than has been thought previously,[6-8] one's impression is that the diet of the local adult Bantu is deficient neither in gross protein nor, presumably, amino acids.

I now propose to comment on lactation, because it is believed by many to be promoted by or even dependent on a greatly increased protein intake, and on liver disease and gynecomastia, both of which are regarded by many as expressions of chronic protein malnutrition.

Lactation

To promote satisfactory lactation, dietary standard bodies recommend increased daily allowances of a number of nutrients, including, *inter alia,* 25 gm. protein, of which one half should be of animal origin. The actual diet suggested for nursing mothers is well known so much milk, meat, eggs, fruit, vegetables, and other such foods. The point to be emphasized here is that Bantu mothers do not get such a diet, yet they lactate very well, producing milk of satisfactory quantity and quality, and often for prolonged periods. Locally,[9] it is believed that there is parity in clinical state and rate of growth between exclusively breast-fed Bantu and white infants up to six months; indeed, Welbourn,[10] also Kark (personal communication), maintain that for this period Bantu babies (among whom self-demand feeding is the rule) grow perhaps more rapidly than do white babies. Quantitatively (at least for the first six months) and qualitatively, therefore, Bantu breast milk must be regarded as satisfactory. Chemically, mean values for the main constituents in Bantu breast milk compare very favorably with those of white mothers' milk.[11, 12] Incidentally, Bantu breast milk has a higher mean protein concentration, namely, 1.35 gm. per cent, than the figure of 1.06 gm. per cent given by Macy[13] for mothers' milk in the United States and 1.19 gm. per cent given by Kon and Mawson[14] for the milk of mothers in Great Britain. Concerning the amino acid composition of the protein, determinations have been made only with regard to methionine and tryptophan; the mean concentrations of both have been found by us to be close to those reported for the milk of white mothers.[15]

It is only fair to point out that among African populations elsewhere, whose diet is lacking in several respects, lactation proceeds less satisfactorily.[16] Nevertheless, it should be emphasized that there are many instances where a measure of breast feeding, occasionally to a surprising degree, has been carried out despite consumption of a poor diet; as, for example, during World War II, at Leningrad during the siege, in starving Holland in 1944, in the Japanese Prisoner of War camps, and even at Belsen Concentration Camp in Germany.[11, 12]

In view of this excellent performance by the Bantu mother, how is she herself affected? First, in regard to the magnitude of the drain sustained by her: during a six-months period she yields about 16 kg. (35 lb.) of total solids. Her own body contains about 18 kg. (40 lb.) total solids. On this particular basis, therefore, during the period mentioned, she gives almost the equivalent of herself in gross nutrients. If she does not eat enough to compensate for this drain, she must lose weight, unless the percentage composition of her body alters. However, she does not seem to lose weight; indeed, our own information suggests that the weight-for-height ratio of such women is possibly greater than that of white lactating women; in veterinary parlance, the successful lactation described is not, therefore, a case of "milking the flesh off the cow's back." Therefore, there can be no doubt that, broadly speaking, the lactating Bantu mother obtains her gross needs of nutrients.

But what of disease—eclampsia, for example? Evidence indicates that Bantu women suffer less than white from this condition. Hipsley[17] considers that a broad correlation obtains between crude fiber intake and eclampsia—the greater the fiber intake, the less the incidence of eclampsia. The Bantu diet is certainly high in crude fiber.

Other comparisons have been made. In Johannesburg, in 1941, Heyns,[18] assisted by Shippel, compared 5059 white women with 1433 Bantu women. These investigators reported that the behavior of the Bantu mothers was superior in all respects. Of the Bantu mothers, 0.56 per cent required operative delivery, as against 5.24 per cent among the white mothers. The maternal mortality of the former was lower. The incidence of stillbirth in the Bantu was 3.5 per cent, a figure less than the corresponding figure of 4.2 per cent for Scotland[19] for the same year. The performance of the Bantu mothers is enhanced in view of the finding recorded by Heyns and Shippel that the Bantu baby, despite a slightly lower weight at birth has a head as big as that of a white baby, yet the pelvic aperture in the Bantu averages about 10 per cent less in area than that of white mothers.

To return to the subject of diet and lactation: one cannot help wondering whether the "vulnerability" of pregnant and lactating women, particularly the former, is perhaps being overstressed. A century ago, among Western populations, large families were usual. I think that it was the demographer Alfred J. Lotka who stated that in the United States, less than a century ago, families including about eight children were almost the rule. In Britain, Sir Walter Scott was a ninth child, Samuel Taylor Coleridge a tenth, John Wesley a fifteenth, and Charles Wesley an eighteenth. Were the nutritional disabilities attached to repeated childbearing and lactation so manifest, relevant information would surely have appeared in contemporary literature or been handed down to us. Admittedly there is much more that one would like to know about the nutrition, biochemistry, physiology, and other aspects of the long-lactating Bantu woman. On the other hand, in spite of this lack of knowledge, two inferences would seem unchallengeable: (1) provided there is a sufficiency of primarily cereal calories, excellent lactation is feasible for prolonged periods; and (2) the failure of the huge proportion of white mothers to lactate satisfactorily can scarcely be ascribed to an inadequate diet. In other words, the role of dietary, as against nondietary factors in human lactation, is overemphasized.

Liver Dysfunction and Disease

In the tropics and semitropics, liver dysfunction and disease are very common among the indigenous populations, and such conditions often are attributed, directly or indirectly, to a low protein intake, or to single or multiple deficiencies of amino acids.[20, 21] One looks askance at this widely accepted etiology because liver disease is common among the Bantu, yet, as stated previously, their diet is characterized neither by a low protein intake nor specific amino acid deficiencies.

Liver dysfunction, insofar as it is reflected by the distribution of the plasma protein fractions and the various precipitation tests on serum, is common in the South African Bantu, being present in more than two thirds of adults examined. Apparently the condition is not improved by the consumption of an adequate diet for protracted periods.[22] Such dysfunction is not accompanied by specific pathology. Concerning liver disease, in a paper by Higginson, Grobbelaar, and myself[23] three types of lesions are described: (1) the fatty liver of kwashiorkor, rarely followed by fibrosis and necrosis; (2) a fine symptomless portal fibrosis, most frequently seen in the second half of life and usually associated with heavy hemosiderin deposition; and (3) a severe cirrhosis, most frequently of postnecrotic origin and showing no specific age trend. More than half of the livers in this third group develop primary malignant change. Briefly, the view is taken that in infancy liver damage is widespread and that, although this damage is not usually recognizable histologically, it is demonstrable by biochemical tests and is undoubtedly caused primarily by malnutrition. Such damage is believed to predispose the liver to the action of factors that precipitate liver disease in adult life. For the local Bantu, it seems probable that such factors include abnormal iron deposition (siderosis), hepatotoxic viruses and, possibly, toxic drugs. There appears to be no adequate evidence that the nutritional factors that so readily induce hepatic damage in small animals (diets, *inter alia*, low in protein) are responsible for the liver lesions noted in Bantu older children and adults

Gynecomastia

This abnormality, enlargement of the male breast, like liver disease, is believed or suspected by many to be an expression, directly or indirectly, of chronic protein malnutrition.[21, 24, 25] Certainly, gynecomastia is common among the Bantu of Southern Africa but, for the reason previously cited, one is not happy over the suggested etiology.

In Johannesburg, at Baragwanath Non-European Hospital (1500 beds), Higginson and Simpson are investigating the incidence of the condition in a series of consecutive post-mortems. Thus far, among 200 adults, they have found a discrete mass exceeding 2 cm. depth in 2 per cent of subjects. At the Witwatersrand Native Labour Association Hospital, also in Johannesburg, about 1000 young Bantu workers (18 to 40 years) from various regions in Central and Southern Africa pass through weekly for medical examination prior to service on the Gold Mines. The incidence of slight or moderate gynecomastia among them is not known, but observations indicate that an average of about one subject per week (0.1 per cent) is known to suffer from *very marked* gynecomastia, that is, having breasts of about 10 to 15 cm. diameter. Such breasts are indistinguishable in appearance and texture from those of young nulliparous women. Since gynecomastia of this type and severity does not appear to have been previously reported, our findings will be described in some detail.

Twenty-one subjects have now been studied by my colleagues and myself. All subjects appeared in good nutritional condition: no evidence of previous dietary privation could be elicited. There was no bias regarding region of origin; that is, gynecomastia did not appear to occur preferentially in subjects from countries in Central Africa, where the nutritional situation is less favorable than in South Africa. Evidence indicated that the gynecomastia developed usually at the time of or within a few years after puberty. Some subjects had been affected for as long as ten years. The enlargement was usually bilateral, nontender, and there was no exudate. These features are in contrast to those observed in the gynecomastia reported to occur occasionally during recovery from malnutrition and undernutrition, for example, among United States servicemen rehabilitated from Japanese P. O. W. camps. Among such affected persons, Klatskin and his co-workers[26] found the mass to be small (1 to 2 cm. diameter) and tender, and sometimes it was possible to express fluid. Two features have been noted about a proportion of the Bantu subjects thus far examined which, provided one is able to establish them satisfactorily, would seem of considerable importance. First, about a third of the subjects maintained that the condition was present in their fathers and occasionally in their brothers, thus introducing a possible familial factor into the etiology. Second, almost all subjects asserted that they were not apathetic regarding their sexual desires, and about a third maintained that they were fathers of children. According to current concepts in endocrinology, such behavior would not be expected. Those who have carried out investigational work among nonwhite people will appreciate the difficulties attached to validating these two findings, particularly the second.

Regarding other features, the hair in axillae and pubes appeared masculine in distribution. Apart from the enlarged breasts, the body configuration was likewise masculine in character. In three quarters of the cases, the span exceeded the height by 4 to 8 cm. In all cases except one the genitals appeared normal. The exception had undescended testes. In all subjects, including the last, the voice had broken. About a third had slight enlargement of the liver; but this feature is not believed to have etiological significance, bearing in mind the equal incidence of hepatomegaly in Africans without gynecomastia. According to the study of Higginson and Simpson already referred to, no correlation is apparent between the presence of gynecomastia and of liver disease (fibrosis, cirrhosis, carcinoma). Furthermore, it may be of interest to add that Higginson and Oettle[27] have found carcinoma of the male breast to be less common among Bantu males than among white males.

Turning now to the biochemical side: briefly, data on serum protein fractions and the various precipitation tests reveal no greater abnormality in subjects affected with gynecomastia than in subjects without the disease. Incidentally, no evidence of liver impairment was found by Klatskin and his co-workers[26] on United States prisoners of war with gynecomastia. A small number of tests for ketosteroids, hydroxysteroids, and ketogenic steroids have shown values lying within normal limits. A limited number of determinations of total urinary estrogens and of component fractions, estradiol, estriol, and estrone, have been made locally by Bloomberg, Higginson, and Keeley (private communication). Thus far, the endocrine picture emerging is nonspecific, that is, the picture in gynecomastia subjects does not differ substantially from that in nongynecomastia subjects. Biochemically, therefore, nothing specific has come to light at least insofar as present studies have revealed.

Regarding the etiology, it will be clear already that the type of gynecomastia seen in the Bantu of Southern Africa, as an entity differs in several respects from the gynecomastia sometimes noted during rehabilitation from malnutrition and undernutrition. The common view that gynecomastia in the African is an expression of habitual protein malnutrition cannot be accepted as established, at least insofar as the local Bantu are concerned. Likewise the view that hepatic disease in these people impairs the normal inactivation of hormones produced by the adrenals and gonads still remains a suggestion. This scepticism does not imply, of course, that the liver is not involved: it merely questions the *modus operandi* suggested. One prefers to consider that injury to the liver and, possibly, to other organs sustained by African infants at weaning time and thereafter may provide a substrate suitable for the development of gynecomastia in later life, such development not being prevented by adequacy of habitual protein intake. The nature of the precipitating factors is not known, although B. J. P. Becker, here at the Institute, has suggested that the estrogenic and other properties of the numerous herbal and other pharmacological preparations used by these people merit serious investigation.

It must be re-emphasized that, in making these and previous comments, adults and perhaps older children are in mind, and *not* the very young. Indeed, it will be appreciated that if only there were a more satisfactory food intake at weaning time and immediately thereafter, there might well be a lower incidence of liver disease and of other diseases later in life. However, the mere provision of milk or milk-substitute foods at weaning time will be insufficient; what will also be required is education in preparing these foods for consumption. In Johannesburg we have found that even when nourishing infant foods are purchased, they are almost invariably made up in far too dilute a form by Bantu mothers.[28]

Fat

The fat intake of the South African Bantu is low; a rough average of about 40 gm. per diem is consumed, providing about 15 per cent of the total calories.[1-3] More than half of the fat is "hidden," being contained in the lightly milled maize products, also in legumes. I refer to three aspects of the fat metabolism picture of these people: (1) the low serum cholesterol levels and the low incidence of severe atherosclerosis; (2) the elevated serum cholesterol levels that are a feature of obese Bantu women; and (3) the high excretion of fecal fat on the "high residue" diet of these people.

Low fat intake, serum cholesterol concentration, and atherosclerosis. No significant difference has been found in mean cholesterol levels of newborn Bantu and white infants.[29] In later life, however, serum cholesterol levels of the Bantu are lower, and the age trend of increase slight in comparison to corresponding data on white subjects.[30] Collaborative current studies show that beta lipoprotein cholesterol concentrations are low, as are also the ultracentrifuge flotation values. Investigation has revealed that severe atherosclerosis in the aorta and in coronary vessels is rare, and that the death rate from coronary heart disease among adult Bantu hospital populations in Johannesburg is about a tenth of that in corresponding United States and Danish hospital populations.[31] A comprehensive study of the chemical composition of total aorta from Bantu and white subjects, in age groups from 20 to 80+ years, has confirmed the far lesser degree of quantitative atheromatous change in the Bantu.[32] The difference in death rates from coronary heart disease in the two racial groups is much greater than the difference in extent of atheroma. In other words, some Bantu do get moderately severe atherosclerosis, yet such lesions, for reasons not yet clear, very seldom lead to occlusion. Incidentally, evidence suggests that among the local Bantu there is no sex bias either of vascular lesions or of death rates from coronary heart disease.[33]

It has been pointed out elsewhere[31, 35] that the low fat intake of the Bantu is but one characteristic of their diet, just as a low incidence of the more serious complications of severe atherosclerosis is but one feature of their disease pattern. It would therefore seem unwise to draw categorical conclusions as to the extent of the relationship involved: it is better at present simply to recognize that the Bantu, living on a diet that is, *inter alia*, low in fat, very seldom die from coronary disease.

However, I now mention a nondietary factor (this, I am afraid, is not a biochemical matter) of possible etiological importance. In a recent paper Mann, Nichol, and Stare,[36] in commenting on the low values found for beta lipoprotein and cholesterol levels in the sera of Nigerians, suggest that a large muscle mass or a large muscle expenditure may well be the effective agent, and that this, and not the diet, is the important element in preventing hyperlipemia and perhaps atherosclerosis. In the South African Bantu, the existence of a large muscle mass—large, that is, as compared with that in the white population—would seem questionable both from anthropometrical and from post-mortem observations. A large muscle expenditure among these people, with the possible exception of Bantu mine workers and a few other groups, is hardly in accord with everyday observations. Obviously, this statement does not mean that these people are lazy, but rather that excessive activity is not characteristic of the Bantu, who, in the past, were primarily a pastoral population. However, there is one feature that might be relevant to the conception of Mann and his colleagues, and that concerns the inexplicably high degree of motor fitness. Thus, using the Harvard step test on groups of somewhat poorly nourished Bantu school children, le Riche *et al.*[37] found that 50 per cent were in excellent or superior physical condition, in contrast to about 5 per cent of 600 children in a private school in the United States similarly rated, as quoted by le Riche *et al.* Similar observations, including other additional tests, have been reported by Morrison *et al.*[38] on Bantu mine workers. More recently, Kloppers and his co-workers, in an unpublished study, compared adult Bantu with corresponding white long-term prisoners on step test performance and found the Bantu to be virtually inexhaustible. A further study of great interest in this regard is that of Gopalan and Ramanathan.[38a] These workers compared fat intake, serum cholesterol levels, exercise, and incidence of coronary heart disease among groups of Indian adults, namely, officers and other ranks of the Indian Defence Force. The fat intake, about 90 to 120 mg. per diem, of the officers was composed of hydrogenated vegetable fat and animal fat (2:1); of the other ranks, chiefly sesame oil (40 per cent linoleic acid). In the former group, cholesterol levels rose from 154 mg. per cent (20 to 29 years) to 201 mg. per cent (40 to 49 years); in the other ranks there was no rise, the mean value being about 145 mg. per cent. The latter group, in contrast to the former, was accustomed to habitual strenuous physical exertion. The incidence of coronary artery disease among the officer class was 23 times that among the other ranks. It might be added that the level of physical fitness of United States recruits in World War II was very disappointing.[39] There can be little doubt that activity and physical fitness are implicated in the etiology of the disease under discussion, although to what extent the feature described has a bearing on the relative freedom of the Bantu and other similar populations from atherosclerosis awaits investigation.

Elevated serum cholesterol concentrations in obese Bantu women. As already stated, the diet of the Bantu seldom is deficient in energy value, cases of severe undernutrition being very rarely encountered. In this section, the point of interest that I wish to emphasize is that, whereas obesity is almost nonexistent in Bantu males, it is very common in Bantu young women, who, moreover, have significantly elevated serum cholesterol values; the causes of both are unknown. Thus, among 50 nonpregnant, nonlactating Bantu women, 20 to 30 years old, all 20 per cent or more overweight when judged by Western tables, the mean serum cholesterol value was 224 mg. per cent. This value is much higher than the figure of 175 mg. per cent found for a like number of nonpregnant, nonlactating, nonobese Bantu women of the same age group. The foregoing would seem to be an accentuated example of the tendency to obesity and slightly raised serum cholesterol values noted by Keys *et al.*[40] among the women of the poorer groups investigated in Madrid, Spain. In the case of the Bantu obese women, according to unpublished observations by Higginson, post-mortem examination of the blood vessels does not reveal atheromatous lesions any greater in frequency or extent than those present in nonobese Bantu women. Here, then, is a problem of interest, a portion of Bantu women who develop obesity and significantly elevated serum cholesterol levels; the latter feature, however, is not indicative of increased atherosclerosis.

High excretions of fecal fat on a "high residue" diet. According to observations on Bantu and white subjects I made some time ago, on a diet containing a modicum of white bread the amount of dry feces accruing per diem is about 20 to 25 gm. When brown bread is substituted for white, the figure rises to about 30 to 35 gm. When the amount of brown bread eaten is increased to about 1 lb. per diem, the yield of dry feces increases to about 40 to 50 gm. With a higher consumption of coarse cereal products, such as quite commonly occurs with Bantu, at least in rural areas, the figure occasionally exceeds 100 gm. dry feces per diem. Enormous stools of this type were also noted by McCance and Widdowson[41] in their *Experimental Study of Rationing*, when their brown bread consumption was about 3 lb. per diem, and were also reported in later studies by these workers when using other high cereal diets.[42, 43] Accompanying this huge increase in stool weight, the time of passage of material through the digestive tract may be reduced to a third of that obtaining on a white bread dietary. Taken together, the changes described, seemingly inherent in altering a diet from one of a "low" to one of very "high residue," may entail as much as a fifteenfold increase in rapidity per unit volume of fecal material traversing the bowel. The relevant changes in intestinal microflora, in biochemistry and metabolism, and various other ramifications appear to have evoked very little interest in this particular field of nutritional science. The special feature to which I draw attention is the excessively high excretion of fecal fat voided under these circumstances. In certain studies of McCance and his co-workers[42, 43] involving the feeding of very high cereal diets to humans, the amounts of fat voided reached 22 gm. per diem, giving an apparent absorption of 54 per cent on the high wheat diet, and 68 per cent on the high oats diet. In two Bantu prisoners whom I observed for 21 days and who were ingesting about 26 gm. fat, the fecal fat voided was found to average about 13 gm. per diem, giving an apparent absorption figure of 50 per cent.[11] In the everyday diet of white populations, a percentage fat absorption figure less than 90 per cent usually is regarded as pathological. Yet the phenomenon described cannot be regarded as of pathological significance, for the extraordinary high excretions of fecal fat are surely the normal response evoked in normal people by the consumption of a diet very high in whole grain or lightly milled cereal products. McCance *et al.*[11-13] incline to the view that much of such fecal fat is composed of undigested fat. Elsewhere,[15] I have suggested that most of such fecal fat is of endogenous origin. I believe that the phenomenon parallels the considerably increased amount of fecal nitrogen voided on "high residue" diets, such nitrogen, as McCance *et al.*[12, 13, 16] maintain, being mainly, if not entirely, of metabolic origin and not merely undigested protein nitrogen. Urbanized Bantu, substituting much maize by white bread, do *not* void such large amounts of fecal fat. The precise bearing of habitually large amounts of fat in the stools on the total body lipid picture is not known. Nor is it known whether the phenomenon described has any bearing on the tardy development of atherosclerosis in these people.

Calcium

It is widely believed that calcium absorption and bone calcification in man are controlled by the available radiation, by the amounts of vitamin D, calcium, and phytate phosphorus ingested, and by the calcium-phosphorus ratio. As already indicated, the high cereal diet of the Bantu is low in ingested vitamin D; with many local population groups the intake is virtually nil. The diet is also low in calcium—the average intake being about 250 to 400 mg. per diem—and has a very adverse calcium-phosphorus ratio. It is certainly high in phytate phosphorus, the intake varying (dependent on the extraction rate of the cereal) but ranging roughly from 1000 to 1500 mg. per diem. Therefore, according to current views the diet of these people, is poor in calcifying capacity. Certainly, there is plenty of sunlight, an average of 8 hours per diem in the Transvaal; but some authorities, such as Hess,[17] consider that a pigmented skin prejudices the production of vitamin D from radiation.

With this background, one would surely expect the stigmata of calcium deficiency to be apparent among these people. In fact, in no respect do the South African Bantu appear to be suffering specifically on account of their usual low intake of calcium.

The skeleton contains 99.5 per cent of the total body calcium. In my laboratory, studies on fifth ribs from Bantu and white subjects have revealed no significant difference between mean percentage composition values for infants and for adults.[18] Determinations of bone density of vertebral bodies (mineral matter per unit volume) have revealed, as with bone composition, a wider range of values than is usually thought to exist, but no significant difference between mean data for both races.[19] Furthermore, samples of vertebral body with marrow removed by refluxing with aqueous ethylene diamine, when observed under the dissecting microscope with regard to mesh, regularity, and trabeculae, showed slight, although not invariable differences between bodies from both races. No bone examined could be regarded as atrophic, and all observations were in harmony with respective mineral density data.

Osteoporosis is not a feature of South African Bantu children or adults.

Concerning fractures, medical officers at hospitals attached to the Gold Mines (which employ about one third of one million short-term Bantu laborers) aver that the Bantu are not more liable to fracture their limbs than are white men under the same stress. These observers also maintain that, accompanying the more rapid callus formation in these people, a Bantu with a fractured leg, when it is healed, can stand on it much earlier than a white person with the same type of injury. These beliefs are only impressions, of course, but they do suggest that the Bantu skeleton, even if it contains, on a height basis, less *total* calcium compared to that of the white man, is not more vulnerable to physical stress or trauma.

Regarding clinical rickets, this disease is common in crowded urban areas in South Africa, just as it is under corresponding conditions at Ibadan in Nigeria,[50] Calcutta and Bombay in India,[51] and elsewhere. But the disease is often observed in breast-fed babies ingesting adequate calcium. Moreover, once the urban Bantu child has freedom of movement and need not be kept largely indoors (that is, after about two years of age), rickets is not seen– at a time when the calcium intake of the child is at its lowest throughout life.[52] In rural areas, however, where much less clothing is worn and where there is no overcrowding, obvious clinical rickets is seldom seen, although the character of the diet, at least in regard to ingested calcium and vitamin D, is much the same in both urban and rural areas.[53] This rarity of severe rickets observed when advantage is taken of available radiation is in agreement with reports from other observers in rural areas; indeed, certain pediatricians of repute almost deny having seen a case of obvious rickets in rural areas in the tropics and semitropics.[54, 55] No case of osteomalacia has been reported in a Bantu woman, pregnant or nonpregnant.

Generally speaking, the teeth of the Bantu, particularly the rural Bantu, are good. In one recent study carried out on children, about 90 per cent were found virtually free from caries; the reverse ratio is believed to obtain with local white children.[56] On the other hand, urbanization, with attendant changes in food habits (increased consumption of such foods as white bread and sugar) shows a deteriorating situation, thereby conforming to a pattern of experience common throughout the world.

Reasons have been given elsewhere for considering that the relationship between calcium intake and the rate of attainment of height or of ultimate height at maturity, is not proved.[57] In any case, studies by Kark and le Riche[58] indicate that the heights of Bantu children compare favorably with those of prewar Central School children of the same age group in Great Britain. Moreover, Bantu mine workers, on an average, are only an inch or so shorter than were British and American recruits in World War II.[57]

Serum calcium values,[59] speaking generally, are about 10 per cent less in the Bantu than in white population groups. Examination of the information on this subject suggests that such values merely reflect a low habitual intake of the element, and are not indicative of low calcium stores.

Perhaps the key to the picture is afforded by the results of calcium balance observations carried out on Bantu prisoners consuming their high cereal, low calcium diet. I found percentage absorptions (apparent absorptions) to be higher and urinary excretions of calcium lower, than among white people on a much higher calcium intake. The combination of these two processes permits calcium equilibrium to be attained despite the low calcium intakes ingested. Other comparable studies demonstrating satisfactory adaptation to low calcium intakes have been enumerated and discussed by Mitchell.[60] In addition, there is the more recent study of Hegsted *et al.*[61]

The question of the "vulnerable" pregnant and lactating woman comes to the fore again. Prior to conception, body calcium of women may be regarded as approximating, roughly, 800 to 900 gm. The calcium content of the newborn infant is about 25 to 30 gm. The loss of calcium sustained by the mother when lactating for 6 months is about 35 to 40 gm., making a total loss of calcium of about 60 to 70 gm., that is, about 7 to 8 per cent of calcium reserves. The actual percentage loss, of course, is much smaller, bearing in mind that throughout the pregnancy and lactation period the mother is certainly absorbing *some* calcium from her diet. Now, a lactating cow can lose as much as 20 per cent of its total supply of calcium without giving rise to deleterious effects or without seriously interfering with milk production.[62] Therefore, on the basis of the information given, the loss of calcium sustained in pregnancy and thereafter cannot be regarded as of serious importance. The additional allowances of calcium usually recommended during pregnancy and lactation perhaps can be regarded as an insurance policy, although the precise risks involved are not altogether clear.

These data strongly indicate that the South African Bantu do not suffer specifically on account of their low calcium intake. Furthermore, the information given, by implication casts doubt on the importance still attached to the alleged deleterious effects of phytate-phosphorus and adverse calcium-phosphorus ratio in human nutrition. The clinical and pathological importance of both these factors, particularly the former, is open to considerable doubt.[63]

It may well be argued: it is conceded that what has been suggested may be valid for tropical and semitropical populations, but what of populations less favored by radiation? With regard to rickets, this argument may be met by citing the studies of Findley and Ferguson[64] on children in Glasgow, Scotland, and of Rustung[65] on children in Norway, where the chief controlling factor was found to be, not the diet consumed, but the regularity with which the child was taken outdoors. Reasons for doubting whether a low calcium intake has any serious relevance to the development of rickets in humans have already been expressed.[66]

Signs of a more realistic approach to the whole subject of calcium requirements and calcium deficiency stigmata are given by McLester and Darby,[67] also Goldsmith,[68] who refer to the lack of methods that permit detection of potential degrees of calcium deficiency: an observation difficult to harmonize with the view often expressed in the past that calcium lack is a widely prevalent condition in the United States. More recently, Hegsted *et al.*[61] boldly averred that calcium deficiency does not occur in adult men. I go still further and raise the question: What is the evidence for *any* syndrome in man shown to arise specifically from calcium deficiency? Conversely, what syndrome in man has been shown to be remedied by calcium supplementation only? Surely, to recommend luxus allowances of a dietary constituent to protect against unspecified stigmata is a highly questionable procedure.

Iron

The Bantu of South Africa are characterized by an extremely high iron intake, often ten times or more the allowances of iron usually recommended. A population whose major source of calories is lightly milled cereal products normally ingests iron in excess of the recommended allowances. In published surveys on the local Bantu, calculated mean daily intakes range from 18 to 57 mg.[1-3] These people, however, derive additional amounts of iron from two extraneous sources: (1) dust and other such substances, and (2) iron derived from the utensils in which they prepare their food. The contribution from the first of these sources is highly variable, depending on the degree of care exercised in food storage, but it may reach one third of the calculated intake. The amount of adventitious iron derived from the second source is far more considerable, the principle of the phenomenon itself having been referred to in papers published by various overseas workers.[69] The chief utensil used by the Bantu is the heavy iron tripod "kaffir pot" (3-l. capacity, weight 4.5 kg.; 10-l., 13 kg.). Such vessels are to be found in almost every kraal in Southern Africa, over one million being sold annually. Iron kerosene cans (18 l., 4 gal.) are also used, especially in urban areas. Moreover, in large-scale cooking for Bantu workers (by industrial undertakings, for example), large iron vessels (100-l. capacity) are also employed. In the cooking of whole maize, maize and "kaffir corn" porridges, beans, vegetables, stew and greens, experimental observations, also determinations in such foodstuffs cooked by the Bantu themselves, reveal an uptake of iron as great as that contained in the parent foodstuffs.[70] Traditional foods of the Bantu, as noted earlier in this paper, include fermented cereal preparations. These foodstuffs, of pH 2 to 3, naturally take up much iron (as much as 10 times that in the raw food) from the preparation vessels. Briefly, our determinations have shown that the iron intake of the South African Bantu often exceeds 100 mg. per diem and, occasionally, reaches and even exceeds 200 mg. per diem.[70] It cannot be too strongly emphasized that it is iron vessels *plus* the particular diet of the Bantu that permit the ingestion of this excessively high iron intake; iron pots used in the dietary context of most Western populations will *not* give rise to a very high iron intake. Most of the adventitious iron is inorganic in character, and therefore readily available for absorption. What is the effect of such high iron intakes, which approximate the involuntary ingestion of several Blaud's pills daily?

Nearly two generations ago German workers such as Queckenstadt[71] had the impression that, with iron intakes much greater than those present in everyday diets, excessive iron was retained, even by subjects not in need of iron. This impression received substantial confirmation from iron balance experiments carried out many years later and, despite the sources of error latent in such studies, they show unequivocally that an abnormally high iron intake is almost invariably accompanied by excessive absorption.[72-76] Less than 10 years ago, Dubach *et al.*[77] studied the absorption of radioiron by 10 normal subjects, total iron intakes being maintained at about 60 to 70 mg. per diem. The data given suggest that 9 of the 10 subjects absorbed a portion of iron that was not utilized in hemoglobin formation, this portion averaging 7 per cent of the iron intake. Unrequired iron was therefore "packed" into the body at the level of iron intake used. Now the only serious avenue of iron loss from the body is by hemorrhage. Briefly then, with iron intakes habitually high, as with the Bantu of Southern Africa, it would seem inevitable that occasional abnormal absorption and retention results, with intermittent deposition and slow accumulation or "overload" of the element occurring in the body.

Abnormal iron deposition or siderosis, is certainly widespread in the Bantu.[78-80] In Johannesburg, about two thirds of adults over 40 years examined at necropsy are affected; in Durban, according to Wainwright,[81] the figure is higher. The deposition in spleen, liver, and vertebral body marrow can and often does reach several grams per cent. The extent of the siderosis does not appear to be related to the cause of death, the condition appearing to be equally common in persons dying in hospital or from trauma.[80] In distribution, the spleen is most affected, then the liver; smaller concentrations are found in the kidney, but only traces in the pancreas, thyroid, heart, and blood vessels (aorta). The distribution thus differs very markedly from that in idiopathic hemochromatosis, particularly in relation to the pancreas, which, in hemochromatosis, is the organ suffering the greatest increase in iron concentration, that is, a mean of 100 times more than normal.[82] Furthermore, diabetes is very uncommon in Bantu siderosis (this is not the case in hemochromatosis) and, although cirrhosis is present in one quarter of severely siderotic livers, the association is far from being invariable.[79,80]

As would be expected, the high iron intake and the siderosis influence certain blood values. There is now a good deal of data, some published,[83, 83a, 84] more unpublished, indicating that iron deficiency anemia among these people is less common than among any other nonwhite population upon which data have been reported. This relationship is understandable, since Rath and Finch[85] have shown that body iron excess cannot co-exist with iron deficiency anemia. In this respect, Higginson,[86] at Baragwanath Non-European Hospital, Johannesburg, in a series of 1500 post-mortems and 500 liver biopsies extending over the last 7 years, has noted only one instance in which siderosis was present in a patient suffering from hypochromic anemia, but whose response to iron unfortunately was not known. Information obtained from this hospital, also from the chief non-European hospital in Durban, both relating to studies still in progress, indicates that incidences of primary iron deficiency anemia in Bantu adults are less than 0.2 per cent of admissions. This very low incidence stands out in startling contrast to a recent study on hospital patients reported from India,[87] in which 63 per cent had a hemoglobin level of less than 6 gm. per cent, and 92 per cent a MCHC less than 32 per cent. Indeed, iron deficiency anemia would seem to be less common in hospitalized Bantu men and women than in numerous nonhospitalized groups of white subjects; for example, in a recent study on 4421 British Royal Air Force recruits,[88] anemia, chiefly of the iron deficiency type (93 per cent), was present in 1.2 per cent of subjects (criteria, Hb. < 12 gm. per cent, MCHC < 30).

Concerning the effect on another component, namely on serum iron, values are frequently elevated in both hospital and nonhospitalized Bantu populations, values occasionally exceeding those reported in hemochromatosis and transfusional siderosis.[83] There are, however, a number of anomalies in the serum iron picture of these people that require elucidation. In one collaborative study,[86] serum iron has been determined in adult subjects who have had liver biopsy undertaken for diagnostic purposes; we have found that excessive hemosiderin, when present in the biopsy sample, was associated with elevated serum iron. The actual level of the latter, however, did not correlate satisfactorily with the intensity of the siderosis. Further work on this problem is proceeding.

The primary cause of the siderosis, one has no doubt, is the habitually high iron intake. The incidence, age and sex affected, the morbid anatomical distribution of the element, and other characteristics are such as might be predicted to arise from habitual iron "overload." Obviously, the abnormal deposition in individuals is highly variable. Not only are there well-known individual differences in biological response, but differences obtain in regard to food preparation, dietary habits, and consumption levels; there are also such factors as blood destruction in malaria and slight hematuria in bilharziasis. Whether the particular pattern of the Bantu diet or malnutrition or undernutrition play any significant role is not yet known.

Finally, concerning the pathogenicity of siderosis, in experimentally induced siderosis in animals the production of pathological sequelae would seem to be almost uniformly negative (data summarized by Higginson *et al.*[80]). At the beginning of the century, authoritative physicians, such as Osler[89] were emphatic that the fact that prolonged iron therapy (extending sometimes for years) was not attended by ill effects. While that may well be valid for white populations, it would seem unjustifiable to assume that the siderosis in the Bantu likewise is innocuous. A liver damaged at weaning time conceivably is more sensitive to insults than one not similarly injured. Earlier in this paper, attention was drawn to the age trend correlation of siderosis and periportal fibrosis in Bantu adults. The extent of the information available on this association does not permit one to dogmatize, but whereas formerly one did not regard siderosis per se as pathological, now one is less sure, and much further work will be required to determine whether siderosis does or does not promote hepatic disease.

Niacin

Goldsmith and his co-workers[90] prepared wheat and maize (corn) diets containing equal, although relatively small, amounts of niacin and tryptophan, 4.7 and 190 mg. per diem, respectively; when fed to humans, only the maize diets produced pellagra. Among the South African Bantu, we have encountered another type of abnormality imposed by a predominantly maize diet, whereby Bantu mothers on a high maize diet have a mean breast milk niacin concentration (70 u gm. per cent), about half that of Bantu mothers on a Europeanized diet (150 u gm.), both diets having an approximately equal niacin content.[91] Whether the abnormality is due to the fact that a large proportion of the ingested niacin is "bound," or to supression of intestinal biosynthesis of the vitamin, or to other causes, is not known. As far as we are aware, there are no stigmata attached to this diminished intake of niacin by Bantu babies; in any case, the usual two thirds cow's-milk mixtures, which have been used successfully for many generations by white populations, contain *less* niacin than that found in the breast milk of Bantu mothers. A further point of interest is that, although Bantu mothers on a diet high in maize ingested only about half the amount of tryptophan present in the diet of Bantu mothers consuming a Europeanized diet, no significant difference was found in the mean tryptophan concentrations in the breast milk from both Bantu groups.

Vitamin C

Early pioneer work on vitamin C requirements was undertaken in Johannesburg by Fox,[92] who drew attention to the very small amounts of the vitamin that could prevent scurvy in Bantu mine workers. Fox's observations received confirmation from later comprehensive studies undertaken on white subjects.[93, 94] That scurvy certainly does occur among Bantu adults was adequately reported by Fox,[92] and two recent South African studies[95, 96] have added to our information on the subject. But the remarkable finding to which one desires to call attention is the extreme rarity of infantile scurvy in Bantu infants (4 cases in Johannesburg non-European hospitals in 5 years) weaned onto cereal "paps," despite the fact that the amount of the vitamin ingested often is virtually nil. The inexplicable fact is that we have nevertheless found usually satisfactory levels of vitamin C in the plasma of such subjects;[97] moreover, other local workers, for example Smit and Pretorius at Pretoria (personal communication) have noted appreciable amounts in the plasma of infants suffering from kwashiorkor. One is confident that large numbers of local Bantu infants receive less than one milligram of the vitamin per diem, yet their plasma levels are consistent with an ingestion of several milligrams per diem. It has accordingly been postulated that under the special conditions of "pap" intake by these Bantu babies, the possibility that there is an endogenous production of vitamin C cannot be excluded. Clearly the phenomenon occurs only in specific dietary contexts; Singh,[98] for example, has reported the occurrence of severe scurvy in Indian infants fed heated cow's milk.

Comment

Perhaps I should apologize for the fact that much of the information I have given refers to aspects other than those of diet and biochemistry as stated in the title of this paper. However, if I had done otherwise, some of the lessons that I am hoping will be learned would have had reduced force.

It will be apparent that one of the main points before me is to emphasize the caution required before considering whether a diet is deficient in certain respects simply because habitual intakes are much lower than the usual allowances recommended. Absence, or at least the apparent absence, of relevant clinical and pathological stigmata in people accustomed to "deficient" intakes cannot but point to the strong probability that the particular recommended intakes are too high. Calcium would seem to be an outstanding example in this field. But the situation regarding animal protein calls for re-examination, as do the needs of pregnancy and lactation.

A further lesson to be learned concerns the biochemical picture (in blood, stools, urine, and breast milk) when it differs in Bantu and white subjects. The lesson, which local workers have thrust upon them, likewise concerns the caution needed before considering that this or that "abnormality" in the Bantu is pathological, when often the "abnormality" is the normal response in healthy persons elicited by the type of diet consumed. Lack of care in this respect would lead one to regard the enviably low blood lipid levels of these people as pathological, whereas precisely the reverse would seem to be the case.

Perhaps the greatest need in this whole subject is to elucidate the extent of the relationship between pattern of diet consumed, of metabolism imposed, and of pathology accruing. In the South African Bantu, the context permits, among other things, of a high rate of mortality and morbidity in the very young, and of a high incidence of liver pathology in later life. On the other hand, however, the context permits, *inter alia*, of a low incidence of certain diseases that, when taken together, exact a high toll of mortality and morbidity from Western populations, notably appendicitis, peptic ulcer, diabetes, cholelithiasis, atherosclerosis, and certain types of cancer. Those of us who are privileged to work in South Africa (although feeling the drawbacks attached to isolation) consider that the best contribution we can make to medical science is to find out all we can about the diet, biochemistry, metabolism, and pathology of its people, in the hope that useful leads of etiological value ultimately may be found, especially in regard to the Western "killers," heart disease and cancer.

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CALORIES AND PROTEIN NUTRITION

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The primary importance of food energy to the maintenance and growth of the living system has long been recognized; in the early days of the development of the science of nutrition the major emphasis was centered upon calories. Data have been and still are being obtained to demonstrate that the energy content of the diet is a critical factor in controlling food intake and that, within physiological limits, animals tend to eat to satisfy energy requirements.[1-5] Consequently, caloric intake is a significant consideration in the study of every branch of nutrition, but a proper balance between energy and nitrogen metabolism is particularly important to the welfare of the individual.[6-9]

The body draws upon protein reserves to correct any deficiency in calories in the metabolic pool for energy.[10-12] Conversely, food energy conserves body nitrogen,[13-18] and there is evidence for an optimum level for caloric intake to keep an efficient balance between fat stores and lean body mass. These optima vary with the age and physiological state of the animal. For example, a weanling puppy requires approximately 200 cal. and 1 gm. of nitrogen/day/kg. of body weight for growth and maintenance. This requirement drops to 0.5 gm. of nitrogen and 125 calories at 10 weeks of age, and then gradually declines to 0.2 gm. of nitrogen/day/kg. of body weight and 80 calories/day/kg. for maintenance during adulthood.[19]

Caloric Intake and Nitrogen Excretion

Much could be written to emphasize the importance of the effect of calories upon protein utilization, but to do this would be to repeat an excellent recent review of this subject by Munro.[18] The following discussion is restricted instead to a consideration of a limited amount of data selected to illustrate some of the general interrelationships between energy intake and nitrogen retention in the body of the animal. The data plotted in FIGURE 1, for example, illustrate the increased excretion of nitrogen that is associated with a reduction in food energy in dogs fed a constant amount of casein nitrogen (3.82 gm./day/m.^{2} body surface area). Rosenthal and Allison[10] demonstrated that the rise in urinary nitrogen excretion that accompanied a reduction in calories was the result of an increase in the excretion of urea. The line dotted through the points in FIGURE 1 is not intended to describe accurately the exact course of the relationship between calories and nitrogen excretion, but it does suggest the tendency to adapt to restriction within a limited range of caloric intakes. Maximum nitrogen retention may not be an ideal measure of the optimum number of calories, since the highest energy intakes with minimum excretion of urea nitrogen can produce obesity. A linear relationship between grams of glucose fed to rats and the nitrogen balance produced over a four-day period was presented by Thomson and Munro.[20] These results also emphasize the influence of food energy upon the nitrogen content of the body of an animal.

Caloric Intake and Body Composition

The effect of varying food energy on the livers of rats fed a constant casein nitrogen intake over a period of thirty-six days is illustrated in FIGURE 2.[21] The energy intake was varied by altering the carbohydrate content of a low-fat diet. The series of white bars in the lower part of this figure record the reduction in the weight of the liver, total protein, and lipid associated with restriction in calories. With protein in the diet, therefore, reduction in calories resulted in a smaller liver, but not in a relative change in composition as expressed in terms of protein and lipid content of the tissue. The heart, the kidney, and other organs of the body were also reduced in size and in total protein in these animals when they were fed a constant casein nitrogen intake, but reduced calories. These results can be interpreted to mean that, in the presence of adequate dietary protein, the rate of growth of these tissues is a function primarily of caloric intake, an interpretation that will be recalled later when data on rates of repletion of protein reserves in adults are considered. The series of bars with slanted lines in the upper part of FIGURE 2 illustrate corresponding values for liver weight, protein, and lipid in rats fed the protein-

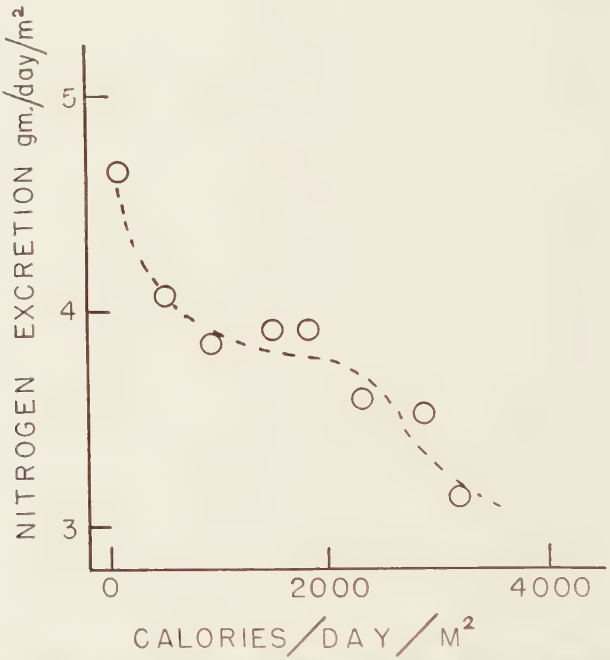

FIGURE 1. Average urinary excretion of nitrogen in 3 dogs fed a constant casein nitrogen intake of 3.82 gm./day/m.^{2} body surface area, but different caloric intakes (data from Rosenthal and Allison[10]).

free diet for thirty-six days. These data demonstrate that liver protein is reduced, but is essentially independent of the caloric intake in rats fed a protein-free diet over this long period. The liver fat, however, increased with the caloric intake so that the ratio between fat and tissue protein was raised markedly by feeding a relatively high caloric intake.

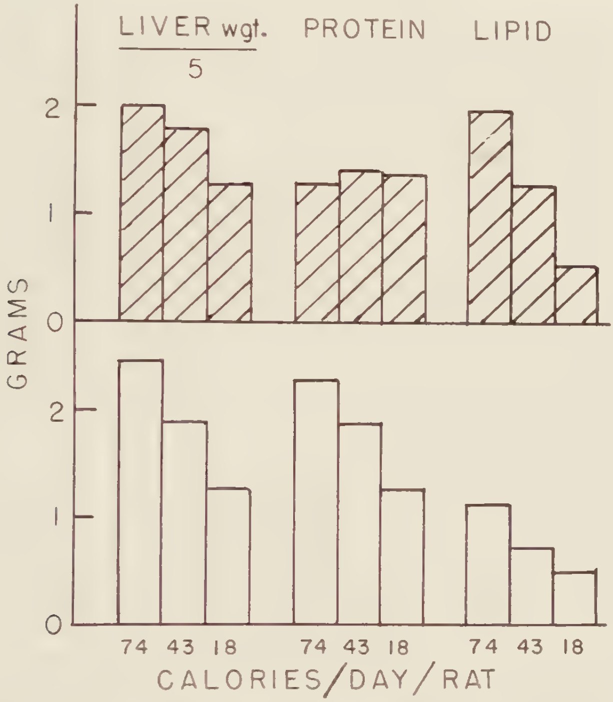

FIGURE 2. The composition of the liver in rats fed a constant casein intake (250 mg nitrogen/rat/day) for 36 days, but with different amounts of calories (the white bars), and in rats fed a protein-free diet with varied caloric intakes (the bars with slanted lines) (data from Rosenthal and Allison[21]).

These data suggest that protein in the diet, even with markedly restricted caloric intake, may conserve some body nitrogen. Such a possibility is illustrated by the data in FIGURE 3.[22]

Caloric Intake and Nitrogen Retention

The correlations between caloric intake and urinary nitrogen excretion and between caloric intake and nitrogen balance in dogs fed different amounts of egg protein are recorded in FIGURE 3.[22] The triangles summarize data obtained while feeding approximately 25 per cent of the daily requirement of calories under the experimental conditions used. The rectangles record data resulting from feeding 50 per cent of the calories, while the circles describe data obtained when the energy intake was adequate. The slope of the line drawn through the circles is called the nitrogen-balance index,[12] which is a measure of the nutritive value of the protein and is a function of the amount of nitrogen retained in the body; the greater the slope, the higher the nutritive value. The slope of this line is unity, a fact that can be interpreted to mean that all of the egg-protein nitrogen was retained for anabolism. The index of the egg nitrogen was still unity in animals fed the diet that contained only 50 per cent of the calories normally required. Even at 25 per cent of the normal caloric requirement, the index was still unaltered at low nitrogen intakes of less than 2 gm./day m.^{2} body surface area, but it decreased rapidly as the nitrogen intake was raised further. Similarly, Cox, Ellingson, and Mueller[23] presented data that pointed to some protein anabolism under restricted energy intakes.

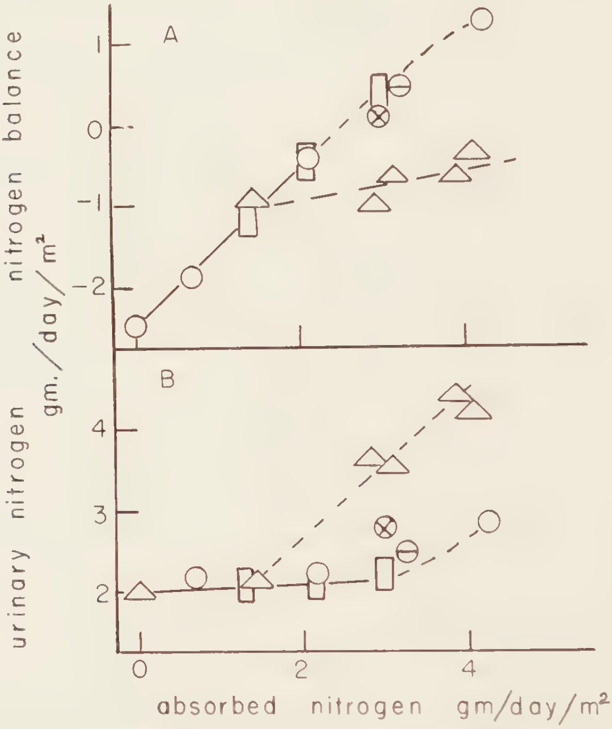

FIGURE 3. Nitrogen balance and urinary nitrogen in dogs as against absorbed egg nitrogen. The open circles record data obtained while feeding full caloric requirements; the rectangles, while feeding 50 per cent; and the triangles, while feeding 25 per cent of the normal caloric intake. The circles with the bars illustrate data obtained by increasing the energy intake from 25 to 50 per cent of normal by adding carbohydrates to the diet. The circles with the cross bars illustrate similar data when the energy intake was increased by adding lard to the diet (data from Allison, Anderson, and Seeley[22]).

A reduction in nitrogen retention in the presence of markedly restricted caloric intakes has been reported in animals and in man under many experimental conditions,[5, 13-16, 24, 25] results that illustrate the principle of a limiting energy level for the utilization of protein by the body. An adaptive process, however, is revealed in the dog by continued feeding of a restricted caloric intake that resulted in a gradual decrease in the excretion of urea nitrogen and a lowering of requirements for calories.[10, 26] For example, an animal with initially full protein reserves was in negative nitrogen balance because of a restricted energy intake, with its concomitant high excretion of urea nitrogen, but continued feeding of the restricted calories resulted in a drift toward nitrogen equilibrium. If the restriction was marked, however, the fat and labile protein reserves were so severely depleted that tissue catabolism eventually increased again, leading ultimately to irreversible damage. Dogs with full protein reserves were shown to adapt to markedly restricted caloric intakes over long periods, while those with restricted reserves drifted more rapidly into the stage of critical loss of body nitrogen.[10]

The circles with the bar in FIGURE 3 record the effect of adding carbohydrate to increase the caloric intake from 25 to 50 per cent of normal. The circles with the cross bars illustrate a similar response while the caloric intake is increased with lard. These data can be interpreted to mean that the nitrogen-conserving effects of carbohydrate and fat calories are equivalent. The data in the literature indicate that this equivalence is generally observed in mixed diets when the conservation is measured over sufficiently long periods of time. Munro and Wikramanayake[27] have called this a nonspecific effect of calories upon nitrogen retention.[28] These authors, however, have pointed out that their data, together with those of others, demonstrate a specific effect of carbohydrate upon protein utilization not observed with lipids. This specific effect is revealed by feeding carbohydrate simultaneously with the protein, a process that results in optimum retention of dietary nitrogen. Studies involving human subjects by Cuthbertson and Munro,[29] those involving animals by Cuthbertson, McCutcheon, and Munro,[30] by Munro,[18] by Geiger, Bancroft, and Hagerty,[31] by Bancroft, Geiger, and Hagerty,[32] by Lathe and Peters,[16] by Thomson and Munro,[20] and by others all demonstrate the improvement of nitrogen retention by feeding carbohydrate and protein simultaneously. Recently Munro[33] has associated this specific effect of carbohydrate upon nitrogen retention with the secretion of insulin evoked as a response to incoming carbohydrate. To quote Munro: "After giving carbohydrate, amino acids are preferentially deposited in muscle and this causes a reduction in their level in the blood. The amino acid supply to other tissues is thus curtailed, with two consequences. First, urea production by the liver is reduced, so that a fall in urinary nitrogen output occurs, and secondly, the tissues other than muscle have a reduced supply of amino acids for protein synthesis. This latter is reflected in the slightly reduced protein content of the liver."[33] Thus, two immediate responses to dietary carbohydrate were observed; one involving utilization of amino acids absorbed after a meal and the other the amino acids liberated from the tissues during fasting (endogenous). To quote further: "Each appears to be the result of insulin called forth in response to the incoming carbohydrate. Evidence has been presented which strongly suggests that the site of this action is confined to muscle, where it would appear that peptide or peptides accumulate temporarily under the action of insulin."[33] (See also Geiger and Pinsky.)[34]

Calories from fat do not appear to have such specific effects upon utilization of protein, although an unusual nitrogen-sparing effect of lipid has been described by Swanson and Clark.[35] These authors reported that a high fat diet depressed the excretion of nitrogen and prolonged the survival period of protein-depleted rats fed a restricted caloric intake. Calloway and Spector[14] found that restriction in calories in normal rats resulted in a negative nitrogen balance that was independent of the fat content of the diet. Similarly, Rosenthal[11] reported that variation in the fat content of the diet did not alter the nitrogen balance indexes of casein fed to normal dogs. The specific effect of carbohydrate, however, was shown by Thomson and Munro[20] to be reduced when fat was substituted for the sugar in a mixed protein diet fed to rats.

Caloric Intake and Rate of Filling of Protein Reserves

It is obvious, from a consideration of these data correlating the effect of food energy upon protein utilization, that many complicated pathways are involved in the phenomenon of the nitrogen-sparing action of calories. In general, however, the net effects of food energy upon nitrogen balance can be illustrated and summarized by the data presented in FIGURE 4.[36] To obtain these data, dogs were depleted in protein reserves by feeding them a protein-free diet for 28 days. The diet contained 80 cal. day kg. of body weight, a caloric intake required by the normal dog fed a mixed diet and living under laboratory conditions. Dogs fed this caloric intake in the protein-free diet were depleted markedly in protein reserves, but they developed fat stores and fatty tissues.

The open circles in the lower part of FIGURE 4 record average data obtained while feeding 0.6 gm. casein nitrogen day kg. of body weight in a diet containing 80 cal. day kg. of body weight to 4 of these protein-depleted dogs. The slope of the dashed line drawn through the circles may be taken as a measure of the rate of repletion of protein reserves to establish nitrogen equilibrium. The line indicates that the dogs came near nitrogen equilibrium within 23 days. The area under the line is a measure of the amount of nitrogen added to the body to attain this condition of equilibrium. The calculated nitrogen retention was approximately 3.2 gm. kg. of body weight, which is low and does not represent the capacity of the body to build up reserves. Continued feeding of 0.6 gm. of casein nitrogen day kg. would probably increase the reserves slowly by maintaining a slight but variable positive balance until the tissue proteins had been developed to a higher level. Increasing the casein nitrogen to 1.1 gm. day kg., but keeping the energy intake at 80 cal. day kg. of body weight, resulted in data illustrated by the black circles and the dotted line. Increasing the nitrogen intake, therefore, raised the nitrogen balance considerably, so that a new condition of equilibrium was approached with much higher protein reserves. Approximately 10 gm. of nitrogen kg. could be estimated to be retained if the feeding period were extended to establish nitrogen equilibrium. The slope of the line, however, drawn through the black circles is the same as the broken line describing the data obtained while feeding 0.6 gm. casein nitrogen day kg. Thus it could be stated that the rate of filling of the protein reserves was the same at both nitrogen intakes, even though the final uptake of nitrogen was approximately three times greater in animals fed the 1.1 gm. than in those fed the 0.6 gm. casein nitrogen/day/kg. The circles with vertical bars record data obtained while feeding 1.1 gm. casein nitrogen/day/kg. of body weight, but with an increased caloric intake averaging 144 cal./day/kg. The slope of the line drawn through these circles is greater than the other two, and nitrogen equilibrium was approached in 23 days, at which time approximately 10 gm. of nitrogen/kg. of body weight had been added to the protein reserves of the animal. The rate of filling of the reserves, therefore, was increased by raising the caloric intake, although the amount of nitrogen retained at equilibrium was mostly independent of the calories. These data emphasize the importance of calories in controlling the rate of filling of the protein reserves of the animal, and they also demonstrate the increased retention associated with a rise in dietary protein intake. The

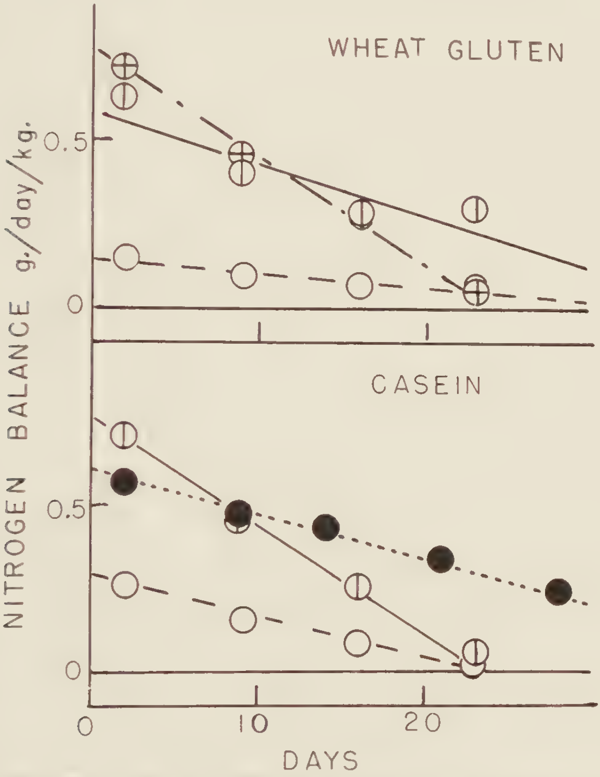

Figure 4. The effect of feeding different amounts of casein nitrogen or wheat gluten nitrogen at various caloric intakes on the nitrogen balance of protein-depleted dogs. These are average data obtained on groups of dogs: 4 to a group (data from Allison[36]).

rate of filling of the reserves, however, is also a function of the pattern of dietary amino acids as well as the caloric intake, a correlation that is illustrated by the data in the upper part of FIGURE 4.

The open circles in the upper part of FIGURE 4 record average data obtained while feeding approximately 0.6 gm. wheat gluten nitrogen/day/kg. of body weight in a diet containing 80 cal./day/kg. of body weight to 4 of the protein-depleted animals. The slope of this line is much less than that describing similar data obtained while ingesting casein nitrogen. The circles with vertical bars describe data resulting from feeding 1.1 gm. of wheat gluten nitrogen/day/kg. in a diet averaging 140 cal./day/kg. Again, the slope of this line is much less than the one describing similar data obtained while feeding 1.1 gm. casein nitrogen/day/kg. at this same caloric intake. Indeed, the slope describing wheat gluten data is similar to the one resulting from feeding 1.1 gm. of casein nitrogen/day/kg., but at a lower caloric intake of 80 calories/day/kg. Wheat gluten is primarily deficient in lysine, so that adding this amino acid increased the rate of filling of the protein reserves to be equivalent to the rate established for casein. The circles with cross bars, for example, illustrate data obtained while feeding 1.1 gm. of the supplemented wheat gluten nitrogen/day/kg. in a diet containing 140 calories/day/kg. body weight. These data, resulting from feeding wheat gluten or casein at various caloric and/or nitrogen intakes to depleted dogs, could be interpreted to mean that the rate of filling of the protein reserves is primarily a function of caloric intake and nutritive value of the protein. The amount of repletion, on the other hand, was markedly influenced by the magnitude of nitrogen intake. Thus the filling of the protein reserves must be described in terms of three dimensions; namely, (1) the nutritive value of the dietary protein, (2) the nitrogen intake, and (3) the caloric intake.[36]

One of the most dramatic illustrations of the importance of balance between these 3 dimensions was developed during an experiment in which groups of puppies were fed the same diet, except that one group received egg protein, another group casein, and a third wheat gluten in the diet. During the fast-growing period the puppies fed egg protein gained 0.5 gm. nitrogen/gm. nitrogen intake, those fed casein gained 0.43 gm. nitrogen, and those fed wheat gluten gained only 0.18 gm. nitrogen/gm. nitrogen intake. All dogs, however, gained essentially the same body weight per gram of nitrogen ingested. The animals fed egg protein were lean and active, while those fed wheat gluten were obese and inactive, thereby exhibiting an inefficient balance between fat stores and lean body mass.[36]

Summary

Data are presented to illustrate some of the interrelationships existing between caloric intake and nitrogen retention in animals. Evidence is given that the body dips into protein reserves to correct a deficiency in calories in the metabolic energy pool and, conversely, that food energy conserves body nitrogen. It is suggested that there is an optimum energy intake to establish an efficient balance between fat stores and lean body mass, and that these optima vary with the age and physiological state of the animal. The initial

response to a reduced caloric intake is an increased excretion of urea nitrogen, but without altering the nitrogen balance index of the dietary protein. Marked reduction in energy intake, however, decreases the retention of dietary nitrogen, results which emphasize the principle of limiting energy level for protein utilization. The data indicate that the body adapts within physiological limits to caloric restriction. Dogs with full reserves can resist a marked lowering of energy intake over a longer period of time than those with low reserves. The rate of repletion of depleted protein reserves in dogs is shown to be a function of caloric intake and of the nutritive value of the protein. Under certain experimental conditions the rate of repletion is a function of caloric intake, and the amount of repletion is a function of nitrogen intake. Thus, the filling of protein reserves must be described in three dimensions, namely, (1) the nutritive value of the dietary protein, (2) the nitrogen intake, and (3) the caloric intake. The importance of a balance among these three dimensions is illustrated by feeding egg protein, casein, and wheat gluten to puppies. The puppies fed egg protein were lean and active, with full protein reserves. Those fed wheat gluten became obese with reduced lean body mass.

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DISCUSSION

William Abbott (*Western Reserve University, Cleveland, Ohio*): The effect of caloric intake on protein requirements and utilization is not only a very interesting problem, but also one of great importance in the recovery of the sick and injured patient. My colleagues and I have followed the work of Allison and his associates; certainly their contributions have been a great stimulus to those of us who must deal with the sick and traumatized patient. It has been largely through his efforts and those of Stare and other workers dealing with basic or fundamental problems in the field of nutrition that the clinician has become aware of the need for an adequate caloric intake in conjunction with protein if good utilization is to be expected.

Until recently, dextrose and normal saline solutions have constituted the only available parenteral fluids employed in the treatment of surgical patients who are unable to eat because of a fistula or obstruction of the gastrointestinal tract, because of peritonitis, or following an injury. About eighteen years ago protein hydrolyzates that could be given intravenously became available and, subsequently, further improvements have been made so that such hydrolyzates are now well tolerated. A better understanding of the need of potassium and other inorganic elements was then reached and, more recently, the provision of supporting calories by intravenous administration of a fat emulsion has been made feasible largely through the contributions of Stare and his associates. Many of the metabolic studies that have been done in the past on the sick or injured patient were limited primarily because such individuals often received diets that were wholly inadequate. One of the problems that has interested us has been that of differentiating the effect of these inadequate diets and the biochemical changes they produce from alterations that have resulted solely from infection, injury, or anesthesia. We have also hoped to determine whether there was any beneficial effect in providing a well-rounded caloric, mineral, vitamin, and nitrogen intake during the acute phase of an illness or following an injury. For the past fifteen years we have been engaged in such studies, and we now feel that it is no longer necessary to argue whether the loss of weight and nitrogen wasting following injury or infection can be prevented or minimized, but whether it is desirable to do so.

Certainly the magnitude of these nutritional deficits is greatly increased in the patient who, for one reason or another, cannot take food. It has also been quite apparent from our studies and from the contributions of others that even the normal individual will lose considerable weight and develop a sizable nitrogen deficit if he is given intravenous feedings or liquid diets similar to those commonly employed in many hospitals today. It is thus apparent that often the metabolic alterations following operation and anesthesia are primarily due to a reduction of appetite and the effects of intestinal ileus or an interference with the normal gastrointestinal function, so that adequate oral nutritional intakes cannot be provided. Our studies indicate that about two thirds to three quarters of the so-called "metabolic response to injury" is in reality comparable to that exhibited by the normal person who is put on a restricted caloric, electrolyte, and protein intake.

Our group has conducted experiments with dogs during the past few years in an attempt to learn whether the additional calories provided by the intravenous administration of a fat emulsion have improved the utilization of ingested nitrogen. These studies were carried out after the dogs had been accustomed to metabolic cages and had been adjusted to a diet of approximately 70 cal. kg. of body weight per day and when their weights had remained constant for a period of at least several weeks. At this point it was believed that they had reached a fairly constant nutritional state, and the studies were begun. Each period was entered on a chart and represented a four-day study. The nitrogen output of all excreta (urine and stool) was determined. The average daily intake for the four-day period was plotted upward from the zero or base line, and the entire nitrogen output plotted downward from the top of the intake column. The area in black above the zero or base line represented a positive nitrogen balance, and any portion of the column extending below the zero line in red indicated a negative nitrogen balance.

During the middle four-day period of these studies the fat was removed from the diet and a comparable amount of a 15 per cent fat emulsion was given intravenously every day. There was a positive nitrogen balance during each of the experimental periods equal to or greater than that seen during the control period that preceded or followed it. In some instances there was also a slight gain of weight during the experimental periods and, insofar as we could determine from these studies, the four healthy dogs utilized the intravenously administered fat emulsion at least as well for a source of calories as a comparable amount of fat taken orally. Two additional dogs were used for identical experiments, except that during the middle periods the entire oral diet was withdrawn and the animals were given a 5 per cent protein hydrolyzate, 10 per cent fructose solution, and a 15 per cent fat emulsion intravenously, in amounts comparable to that consumed during the control periods by the ingestion of oral diets. It can be seen that these animals did almost as well during the periods they received all nutrients intravenously as they did in the control periods when their intake was consumed orally. During the time that the intravenous alimentation was being provided, these dogs showed nitrogen equilibrium or a slightly positive nitrogen balance.

We have also studied thirty male patients during the past five years. These patients all had a gastric operation for ulcer of the duodenum or stomach. Prior to operation these patients were considered to be relatively healthy individuals. They had not had any recent vomiting or bleeding, so that, insofar as it was possible to determine, they were all brought to the operating room in as near a comparable nutritional state as was feasible. There were variations in their ages and physical stature that obviously could not be avoided. These patients were studied after being started on a diet for several days prior to the operation. This group of 14 patients received only a 5 or 10 per cent hexose (dextrose or fructose) solution. These solutions provided approximately 500 to 1200 calories daily and contained no nitrogen. On the average, these patients showed deficits of approximately 58 gm. of nitrogen, and their deficits ranged between 50 and 70 gm. of nitrogen over this 5-day period. Although their weight loss varied somewhat from one individual to another, there was approximately 1 lb. to 1½ lb. of weight lost per day. The patients who were given a comparable sugar intake but, in addition, received approximately 0.2 gm. of nitrogen daily per kilogram of body weight showed a 30 to 40 gm. nitrogen deficit over a comparable 5-day postoperative period, with an average deficit of about 35 gm. of nitrogen for the 5 days. Their weight loss was almost half that exhibited by the group of patients who received only a hexose solution for the 5 days. The third group of patients received a comparable or slightly greater nitrogen intake but, in addition to the sugar and protein, they were given a fat emulsion, so that their caloric intake was increased from the 1200 to 1700 cal. received by the preceding group to a point between 2000 and 3000 cal. per day. This provided approximately 35 cal. kg. of body weight per day. It is evident that the weight loss and nitrogen deficits were greatly minimized. This latter group of patients either lost or gained only 1 or 2 pounds of weight and showed an average 5-day cumulative postoperative nitrogen deficit of 8 gm.

Balance studies of some of this group of 30 patients demonstrate the fact that the patients who received about 0.2 gm. of nitrogen per day and 150 to 300 gm. of carbohydrate lost between 30 and 40 gm. of nitrogen during the 5-day period. This deficit and the loss of weight could also be minimized by giving an anabolic hormone, Depo-Testosterone propionate or Depo-Nortestonate. By giving one injection of 250 mg. of this hormone several days prior to operation it was noted that the nitrogen deficit and weight loss could be reduced by approximately 50 per cent.

Studies of two women who also underwent a subtotal gastrectomy emphasize the fact that the magnitude of the metabolic response of the female is about one half that exhibited by the male undergoing a similar operative stress. It is believed that this variation is due partially to the greater amount of fat and lesser amount of muscle in women and to a somewhat different endocrine response.

Two other cases also underwent a subtotal gastrectomy. A man who was given 3000 ml. of a 10 per cent dextrose solution daily showed a 61-gm.-nitrogen deficit over the first 5 days after his operation and a 6-kg. weight loss. The other patient, who underwent a comparable operation and was given adequate amounts of protein, sugar and fat intravenously, showed no loss of weight, and his nitrogen deficit was almost nil, being approximately 3 gm. over the first 5 postoperative days.

I believe it is also worth emphasizing that many of these patients who had had a subtotal gastrectomy could not eat adequately during the postoperative period, so that frequently studies reported in the past that failed to show a prevention or minimizing of the weight loss and nitrogen wasting were in reality a reflection of the inability to provide an adequate caloric intake. I emphasize the fact that, although the patient, his relatives, or the nurse may all tell us that the patient is having a well-rounded diet, our studies indicate that often, when we have an opportunity of making an accurate investigation, the amount of food actually consumed usually proves to be much less than that ordered by the physician and considerably less than the amount the patient feels he is having. In other words, the physician must determine how much of the diet that he has ordered is actually being consumed before he can conclude that the loss of weight and general debilitation that result following an operation or injury are due to the trauma per se rather than to inadequate nutritional intake. It has also been frequently stated that protein, fats, and other nutrients are not utilized in proper fashion following an injury. However, most of the studies done do not support this view, and the evidence available seems to indicate that there is probably a normal utilization of protein and other nutrients but that, under certain circumstances, an acceleration of the rate of utilization or turnover may occur.

In summary, I emphasize again that much remains to be learned in the field of nutrition as it relates to the care of the sick and injured patient. Our studies indicate that persons who are undergoing a major operation or who have experienced severe trauma should be given approximately 0.3 gm. of nitrogen and 40 cal. kg. of body weight per day. Women may often do well on somewhat lesser amounts. Little is known concerning the specific individual amino acid requirements for the injured or ill patient, but undoubtedly future studies will clarify this problem and others.

We now believe that many of the alterations reported to occur following an injury have been, in reality, primarily the result of starvation. Obviously it is often not advisable to feed injured and desperately ill patients forcibly, but it also should be remembered that starvation regimens likewise have no place in the modern treatment of the sick and injured patient. We certainly agree with Allison that it is important to support a good protein intake with an adequate amount of calories.

T. B. Van Itallie (*Harvard University School of Public Health, Boston, Mass.*): It is not uncommon for physicians to encounter patients who are unable to consume enough food to remain in calorie and nitrogen equilibrium. When this occurs, a decision must be made as to what combination of nutrients will be optimal under the circumstances.

In attempting to decide what to feed such patients (whether this is to be accomplished by mouth, stomach tube, or parenterally), the physician must draw upon basic information about calorie-nitrogen relationships such as that obtained by Allison and his colleagues in experimental animals. Indeed, we are grateful that work of such quality is available to us for, were we forced to rely on studies done on human subjects, we should certainly feel far less secure in enunciating certain principles of supportive nutritional care.

This is partly because most nutritional experiments on man are too brief and, as we see from the figures illustrating the paper under discussion, we cannot interpret any one phase of a nutritional experiment without reference to the other phases, before and after.

As a result of such studies we are beginning to be aware of certain important factors that must be considered when we attempt to make the best nutritional "compromise" possible.

In general, the objective of supportive nutrition is to build up or maintain protein stores, or to minimize their loss, depending on the clinical situation. Toward the attainment of this end, the three dimensions discussed by Allison are of importance: namely, the quality of the ingested protein, the level of the nitrogen intake, and the level of the calorie intake. In addition to these dimensions there are several others that deserve mention. For example, study of the role of carbohydrate should be given a high priority.

In the nondepleted experimental subject, a number of investigators have shown that 50 to 100 gm. of carbohydrate, administered in the absence of all other nutrients, will forestall ketosis effectively and will diminish nitrogen and associated electrolyte losses by almost one half. Obviously, carbohydrate does not accomplish these results by virtue of its ability to supply calories. It would seem that this quantity of carbohydrate is enough to replenish the oxaloacetate lost by attrition in the Krebs cycle and to provide some of the glucose needed by the nervous system.

In the absence of small quantities of carbohydrate from outside sources, particularly when liver glycogen stores are exhausted, an excessive rate of gluconeogenesis from protein occurs, leading in turn to a marked nitrogen deficit.

Thus, carbohydrate spares protein in two ways: (1) as an essential nutrient needed for what Peters[1] has called "operative" metabolism—as a part of the "machinery" of metabolism, and (2) as a fuel. When carbohydrate and fat are considered as fuels they are interchangeable, as Allison has pointed out, but for operative purposes this is not the case.

The same 50 gm. of carbohydrate that afford protection against ketosis and excessive nitrogen loss provide only 200 cal. This amount may be one tenth of the caloric needs of the patient. Much of the remainder can be met by drawing upon the fat depots. However, there is always some protein broken down to provide calories and, in the absence of an adequate intake of calories and protein, some degree of nitrogen deficit is bound to occur.

In nutritional situations in which one must compromise on quantity, how can this deficit be kept at a minimum? Allison has provided us with some clues, and Calloway and Spector[2] have reviewed the nutritional literature on this subject with interesting results.

I propose to mention several of their conclusions, conceding that we are in a controversial area. When the adult male is deprived of all food, the nitrogen deficit early in starvation is about 12 gm. day. When the calorie intake is raised from 0 to 700 from nonprotein sources, it is possible to reduce this deficit to about 7 gm. day. According to Calloway and Spector, a subsequent increase of calorie intake up to levels of 2800 calories seems to be without further advantage in the sparing of body protein. When not more than 400 to 600 cal. are supplied from nonprotein sources, there seems to be no advantage in administering protein. However, it is important to decide whether, in parenteral nutrition, one should or should not administer amino acids when the calorie intake is limited to 500 cal. Nevertheless, according to Calloway and Spector and others, protein ingested under such circumstances is largely burned as an energy source. If the level of nonprotein calories is raised to 900 cal. day, according to Calloway and Spector, any excess over 3 gm. of nitrogen per day is virtually of no further advantage. Whether these data are precisely accurate or not is not too pertinent at this time, since many more studies must be done on patients at various stages of repletion and depletion before we can be sure of the effect of a changing nutritional status upon these relationships. However, we must discover what we can about this subject, because without appropriate information we cannot rationally plan a program of compromise feeding. We should like to know whether it makes sense to administer amino acid preparations to a patient whose calorie intake is only 600 or less.

Plough and his associates[3] have summarized the relationship between calories and nitrogen by pointing out that the magnitude of the protein-sparing effect of added calories increases with an increase in the level of dietary protein. I believe this relationship is also illustrated in Allison's studies. However, such a rule may not hold if the calorie intake itself is severely restricted; according to the work done on rats by Vitale and his associates in our department, there are some suggestions that, at a submaintenance calorie intake, protein feeding can be detrimental.

If we grant that these general principles about calories and nitrogen in depleted and nondepleted individuals have some validity, there are still several considerations that need further explanation. For example, fatty tissue is supposed to contain 1.1 per cent nitrogen. Does this mean that when a calorie is stored as fat, 1.8 mg. of nitrogen also must be stored? If this is the case (and Plough *et al.* have suggested that it may be), how are we to interpret the positive nitrogen balances associated with very high calorie intakes, when fat is being laid down? Actually, although fatty tissue contains some protein, it seems unlikely that the triglyceride stored in the fat cells has to carry with it nitrogen in the same proportion.

The topic of positive calorie balance leads us naturally to a consideration of surfeit feeding. Is it desirable to maintain nitrogen stores at their physiologic maximum, or can we have too much of a good thing? We need to learn more about the desirable proportion of stored fat to stored nitrogen. In this regard, can we equate the consequences of surfeit feeding with optimal health? As Allison has pointed out, we cannot do so. It would seem that more work must be done to correlate nutrition with functional capacity. We need to know whether the rapid repletion of protein stores obtainable by feeding of a high-calorie diet has clinical merit, and we also need to know whether increased storage of protein in the body has clinical value.

I should like to summarize what I have said by repeating my conviction that the clinician needs sound nutritional principles to guide him in his planning of nutritional supportive care. Studies such as those of Allison are very useful in helping to provide a rational basis for such principles. Nevertheless, the physician still must determine the extent to which a discernible clinical advantage accompanies increased rates of protein repletion, or a degree of protein storage larger than usual.

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BALANCE AND IMBALANCE OF AMINO ACIDS

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Recognition of the importance of the proportions of individual amino acids in dietary proteins can be traced to the classic investigations of Willcock and Hopkins[1] and of Osborne and Mendel[2] about fifty years ago. These pioneer studies, which gave rise to the concept of dispensable and indispensable amino acids, indicated clearly that the nutritive value of a protein was related to its amino acid composition. These observations led to extensive investigations by many nutritionists, culminating in 1935 in the isolation of threonine by McCoy, Meyer, and Rose.[3] This discovery, together with the previously accumulated knowledge, made it possible to estimate quantitatively the amino acid requirements of several species and, as methods of determining the amino acid composition of proteins were improved, to relate differences in the nutritive value of proteins to differences in their content of indispensable amino acids.[4, 5] These developments paved the way for detailed studies of interrelationships among amino acids, and between amino acids and other dietary components, thus leading to an awareness of the importance of amino acid balance and imbalance in nutrition.

Amino Acid Balance and Imbalance

The multitude of studies on amino acid requirements and deficiencies and on the effects of amino acid supplementation have made it clear that, in order to support optimum growth, a diet must provide each indispensable amino acid in the quantity in which it is required by the animal.[6, 7] Although requirements vary with the age and condition of the animal[7, 8] and may be affected by a number of other factors,[7] it is possible, for a given set of conditions, to calculate a ratio representing the relative requirements of the individual indispensable amino acids.[4, 5] Ratios of the requirements calculated from biological assays have been shown to correlate fairly well with those estimated from carcass analyses.[9, 10]

An idea of the nutritional adequacy of a protein can be obtained from a comparison of the ratio representing the relative proportions of the indispensable amino acids in a protein with that representing the relative amino acid requirements of the animal. Difficulty is encountered in satisfying the requirements of the animal if the ratio of amino acids in a protein deviates appreciably from the ratio of the requirements. The unsatisfactory rate of growth of animals consuming nutritionally unbalanced proteins is frequently attributed to an amino acid imbalance. Apart from the fact that it is necessary to provide amino acids in certain definite proportions in order to meet the individual amino acid requirements, our knowledge of the limits within which the balance of amino acids in different diets may be varied without leading to ill effects is limited and is undoubtedly influenced by many factors. Certainly, growth is suboptimal when one or more amino acids are provided at levels below the requirement. However, this suboptimal growth is not attributable, as a rule, to the amino acid balance per se, but to the fact that the diet is specifically deficient in an essential component, just as the poor growth of an animal receiving a diet low in thiamine is not directly attributable to poor vitamin balance, but to a thiamine deficiency. There are, in contrast, cases in which it can be demonstrated that a change in the proportions of the amino acids in a diet, quite apart from the direct creation of a deficiency, can cause an adverse effect on growth or some other metabolic process. Thus the use of the term imbalance as a synonym for deficiency, as is not infrequently done, can be misleading.

At this point I propose to stress the difference between the two prefixes "un" and "im." "Un" carries merely the meaning of negation or not, and the term "unbalanced," meaning "not balanced," is useful for describing proteins that are low in one or more essential amino acids or that are generally poorly balanced from a nutritional viewpoint. This would be in accord with the dictionary definition. Diets containing unbalanced proteins give rise to the development of amino acid deficiencies. On the other hand, the term imbalance will not be found in the average dictionary, and it usually carries a specific medical connotation when so listed. However, since the prefix "im" can be used to connote "intensive negative force," the term would be a stronger term than "unbalanced," and it would imply a more severe condition or even a somewhat different type of condition. There is, therefore, a good case for reserving the term "imbalance" for situations in which an adverse effect, more severe than the original deficiency, results from an alteration in amino acid balance per se, one that can be prevented by correcting the balance.

Since any alteration in the amino acid composition of a diet, other than a proportional change in all the amino acids, alters the amino acid balance of the diet, it is not surprising that adverse effects resulting from additions of amino acids to diets under a variety of conditions have been attributed to the creation of amino acid imbalances. Growth retardations observed when animals have ingested excessive amounts of certain individual amino acids have been attributed to amino acid imbalances; the greatly reduced weight gains of animals receiving vitamin-deficient diets to which large amounts of individual amino acids have been added have received the same attribution; increases in the severity of certain vitamin deficiencies following an improvement in the nutritive value of the dietary protein have been cited as examples of effects of such imbalance; the same thing has been said of growth retardations resulting from the addition of various amounts of amino acids or nutritionally unbalanced proteins to diets that are primarily deficient in an amino acid; and the term has also been used, as indicated earlier, synonymously with amino acid deficiency.

A short time ago it appeared plausible to classify all of the deleterious effects of dietary additions of amino acids under the general heading of amino acid imbalance.[11] However, as information has accumulated, it has become increasingly difficult to justify this procedure,[12-15] and it now appears that there are at least 2 or, possibly, 3 distinct types of such adverse effects. It is accordingly necessary, before going further, to propose a definition for the term "amino acid imbalance" in the hope that, if the definition is not generally accepted, it will at least serve as a basis for discussion.

I suggest that the term "amino acid imbalance" be used to describe those cases in which the addition of a relatively small amount (what might be called a supplemental amount) of an indispensable amino acid, or of a mixture of such amino acids, or of an unbalanced protein to a diet that is low in one or more amino acids causes a retardation of growth or some other adverse effect that can be prevented by the concomitant addition of small quantities of the limiting amino acids to the diet. The critical points are that the diet must contain a marginal amount of at least one indispensable amino acid, and that the effect of the imbalance can be prevented by a small supplement of the most limiting amino acids in the diet. In essence, this would be the creation of a more severe amino acid deficiency through an alteration in the amino acid balance.

If this definition is accepted, it then becomes necessary to distinguish between imbalances and other adverse effects of amino acid additions.

Amino Acid Toxicity

Detrimental effects that result from the ingestion of excessive quantities of vitamins are attributed to vitamin toxicities, so it would appear logical to attribute the detrimental effects that result from the ingestion of excessive quantitites of individual amino acids to amino acid toxicities. Although many amino acids become toxic under some conditions,[16] it is unlikely that the levels required for such effects to become evident would ordinarily be encountered. The results listed in TABLE 1 illustrate growth retardations that have been observed with excessive quantities of some amino acids. The diet used in these experiments was a complete purified diet that supported a good rate of growth when the protein level was adequate. Young rats were used in the experiments, which were of 2 to 3 weeks' duration. It is evident, even from these few examples, that the toxic levels of the various amino acids differ greatly.

In some cases these toxic effects have been reversed, while in others various attempts to overcome the toxicity have been unsuccessful. The toxic effects of a dietary excess of methionine can be counteracted by increasing the intake of arginine and glycine[17] or by providing glycocyamine[18] in the diet, as illustrated in TABLE 2. These compounds might almost be considered as antidotes for methionine poisoning. The severe effects of an excess of leucine in animals fed on a low-protein diet can be prevented by the inclusion of more isoleucine[16] or isoleucine and valine[19] in the diet, as shown in TABLES 3 and 4. This growth retardation appears to be the result of an amino acid antagonism. It is not prevented by a mixture of the most limiting amino acids in the diet, but only by the structurally similar isoleucine and valine. Wretlind[20] has recently observed that the utilization of D-valine is similarly prevented by an excess of D-leucine.

There is no evidence of a common basis for dietary amino acid toxicities. Each appears to be different, and it seems probable, as Russell, Taylor, and Hogan[21] have suggested, that these effects are inherent properties of the individual amino acid molecules or their breakdown products. The toxicity may be more severe when it is superimposed on an amino acid deficiency. Also, there are differences in the effects of the D and L isomers.[16, 22, 23] The limited evidence available suggests that excessive quantities of amino acids are less toxic if the diet contains an adequate supply of all amino acids.[24] For example, the growth of rats receiving levels of casein as high as 60 per cent is not appreciably retarded; yet a diet with this level of casein would contain about 1.9 per cent of methionine, 4.5 per cent of lysine, 5.4 per cent of leucine, 3.5 per cent of tyrosine, and high levels of all amino acids except tryptophan and cystine. In general, dietary amino acid toxicities have not been studied methodically,[16] and observations on their effects on the concentration of free amino acids in the blood have not been illuminating.[25, 26]

TABLE 1

EFFECT OF INGESTION OF EXCESSIVE QUANTITIES OF INDIVIDUAL AMINO ACIDS ON GROWTH OF RATS RECEIVING ADEQUATE AND INADEQUATE LEVELS OF PROTEIN

Amino acid addition	Rate of gain	
	9 per cent casein, gm./week	18 per cent casein, gm./week
None	15	33
2.5 per cent DL-methionine	2	17
3.0 per cent DL-tryptophan	5	22
4.0 per cent L-lysine HCl	9	25
3.0 per cent L-leucine	2	32
3.0 per cent DL-aspartic acid	10	26

TABLE 2

EFFECT OF ARGININE AND GLYCINE IN PREVENTING THE GROWTH DEPRESSION CAUSED BY EXCESS METHIONINE (250-GM. RATS DURING 20 DAYS)[17]

Diet				Change in weight, gm.
Casein, per cent	Methionine, per cent	Arginine, per cent	Glycine, per cent	
12	—	—	—	7.4
12	4.8	—	—	−36.6
12	—	—	4.8	1.2
12	4.8	—	4.8	−1.0
12	4.8	1.7	4.8	−5.0
12	—	4.8	1.7	−5.1

Amino Acid-Vitamin Relationships

The effect of an excess of an amino acid or amino acids in creating a more severe vitamin deficiency might be considered as a special case of amino acid toxicity. Such effects have been demonstrated in animals fed on diets deficient in niacin, pyridoxine, or vitamin B_{12},[16] all of which have been implicated in amino acid metabolism. An example is given in TABLE 5. Except when the levels used have been such that a true toxicity has been superimposed on the initial effect, these can be reversed by providing an adequate amount of the missing vitamin. The growth retardation in such cases is evidently the result of an increase in the rate of catabolism of the particular vitamin. This is further suggested by the observation that an increase in the level of a nutritionally well-balanced protein can increase the severity of certain vitamin deficiencies.[28]

TABLE 3

EFFECT OF PROTEIN AND AMINO ACID SUPPLEMENTS ON THE GROWTH-RETARDING ACTION OF L-LEUCINE

Diet				Weight gain, gm./week
Casein, per cent	L-Leucine, per cent	DL-Isoleucine, per cent	Other	
9	—	—	—	17 ± 1.3
9	3.0	—	—	2 ± 1.0
18	—	—	—	35 ± 1.0
18	3.0	—	—	34 ± 2.0
9	—	—	A.A.s*	24 ± 1.3
9	3.0	—	A.A.s*	4 ± 1.5
9	—	0.5	—	16 ± 1.5
9	3.0	0.5	—	12 ± 1.0

* Amino acids: 0.1 per cent DL-tryptophan, 0.6 per cent DL-threonine, 0.6 per cent DL-valine, 0.3 per cent L-histidine HCl, 0.5 per cent L-lysine HCl, and 0.3 DL-phenylalanine.

TABLE 4

EFFECT OF ISOLEUCINE AND VALINE IN COUNTERACTING THE GROWTH-RETARDING ACTION OF EXCESS L-LEUCINE IN RATS FED 9 PER CENT CASEIN DIETS SUPPLEMENTED WITH AMINO ACIDS

Diet	Average weight gain, gm./week
Nine per cent casein + essential amino acids*....................	18.4 ± 1.3
Basal + 3 per cent L-leucine..................................	1.9 ± 0.7
Basal + 3 per cent L-leucine + 1.2 per cent DL-isoleucine..........	7.8 ± 0.5
Basal + 3 per cent L-leucine + 1.2 per cent DL-isoleucine + 1.2 per cent DL valine	15.3 ± 1.3

* Essential amino acids included 0.1 per cent DL tryptophan, 0.3 per cent DL methionine, 0.54 per cent L-lysine, and 0.7 per cent DL-threonine.

The creation of a more severe vitamin deficiency by improving the dietary protein and increasing growth, as has been shown in choline deficiency,[29] certainly demonstrates the importance of nutritional interrelationships, but it cannot be considered as an effect of amino acid imbalance. This case is complicated by the role of methionine in the biosynthesis of choline. Nevertheless, it is analogous to the masking of a B-vitamin deficiency by feeding an amino acid-deficient diet, which retards growth to such an extent that the vitamin deficiency does not become evident until the dietary protein is improved. In neither case, if the vitamin is provided, can any adverse effect from the amino acid or protein supplement be demonstrated.

Amino Acid Imbalance

To return to the subject of amino acid imbalance as defined earlier: the first example that falls within the limits of the above definition is the growth retardation observed by Krehl, Sarma, and Elvehjem[30] upon adding gelatin to the diet of rats receiving suboptimal amounts of tryptophan and niacin. A similar growth retardation was induced when an amount of threonine equivalent to that contained in 6 per cent of gelatin was included in the low-protein (9 per cent casein) diet supplemented with cystine or methionine.[31, 32] The addition of either niacin or tryptophan prevented the growth retardation. The reversal by niacin was apparently due to its ability to spare tryptophan.[33] Salmon[34] and Sauberlich and Salmon[35] were able to induce a growth retardation that could be prevented only by the addition of tryptophan. The results shown in TABLE 6 illustrate some of these observations. No imbalance could be induced unless the diet was supplemented with cystine or methionine, thus making tryptophan limiting for growth. In this case the addition of threonine depressed the growth rate below that obtained with 9 per cent of casein without

TABLE 5

EFFECT OF EXCESS METHIONINE ON THE GROWTH OF RATS RECEIVING VARIOUS LEVELS OF PYRIDOXINE (DIET CONTAINS 18 PER CENT OF CASEIN AND SUCROSE)[27]

Additional DL-methionine, per cent	Grams gained in 4 weeks, pyridoxine (mg./kg. diet)		
	0	0.6	6.0
0	34	78	121
0.4	17	85	131
1.0	5	25	114
2.5	—	3	59

TABLE 6

INTERRELATIONSHIPS AMONG METHIONINE, THREONINE, AND TRYPTOPHAN IN THE CREATION OF AN AMINO ACID IMBALANCE IN RATS RECEIVING 9 PER CENT OF CASEIN (NIACIN OMITTED FROM VITAMIN MIXTURE)

Diet				
Casein, per cent	DL-Methionine, per cent	DL-Threonine, per cent	DL-Tryptophan, per cent	Weight gain, gm./week
9	—	—	—	10
9	0.3	—	—	12
9	—	0.36	—	10
9	0.3	0.36	—	4
9	0.3	0.36	0.1	20

any supplement. Thus, the addition of threonine, which was not limiting for growth, to a diet deficient primarily in tryptophan produced a growth retardation readily corrected by supplying the limiting amino acid; namely, tryptophan. Henderson, Koeppe, and Zimmerman[36] have demonstrated that similar imbalances can be produced with supplements of lysine and valine when these amino acids are the second most limiting amino acids for growth.

In studies on liver fat deposition utilizing a similar diet containing niacin and choline, it was observed that, although tryptophan may be limiting for growth, threonine is limiting for the control of liver fat deposition.[37, 38]

TABLE 7

EFFECT OF METHIONINE ON GROWTH AND LIVER FAT OF RATS FED ON 9 PER CENT CASEIN-SUCROSE DIET

Diet			Weight gain, gm./week	Liver fat, per cent wet weight
Casein, per cent	DL-Methionine, per cent	DL-Threonine, per cent		
9	0	0	11	10.5
9	0.3	0	16	28.2
9	0.3	0.36	20	12.0

TABLE 8

EFFECTS OF AMINO ACID SUPPLEMENTS ON GROWTH OF RATS FED ON LOW-PROTEIN DIETS CONTAINING EGG ALBUMIN

Diet					Rate of gain, gm./week
Egg albumin, per cent	DL-Threonine, per cent	DL-Valine, per cent	L-Lysine HCl, per cent	L-Histidine HCl, per cent	
8	—	—	—	—	8.6
8	0.36	0.2	—	—	14.9
8	0.36	0.2	0.5	—	9.7
8	0.36	0.2	0.5	0.25	14.2

In this case, as shown in TABLE 7, the addition of methionine to the 9 per cent casein diet caused fatty infiltration of the liver, apparently by creating a more severe deficiency of threonine.[39] This could be very largely prevented by adding threonine together with methionine.

A number of examples of growth retardations that appear to have a similar basis have been observed. The addition of lysine to a low-protein diet containing egg albumin caused a growth depression in rats that was largely prevented by a supplement of histidine (TABLE 8).[10] In a study on amino acid supplementation of rye flour, Sure[11] found that the addition of threonine or threonine and lysine, which were not limiting for growth, caused a reduction in the rate of growth. Gessert and Phillips,[12] working with dogs, demonstrated imbalances involving lysine and methionine when these two amino acids were about equally limiting for growth. A similar effect of lysine has been observed in rats fed on diets containing soybean proteins[13] and in pigs fed on corn-cottonseed meal[14] or corn-soybean meal diets.[11, 45] Pecora and Hundley[46] and ourselves,[47] during studies on the deposition of liver fat, observed that various combinations of amino acids will produce amino acid imbalances that cause a depression of the growth rate of rats maintained on rice diets (TABLE 9). Richardson, Blaylock, and Lyman,[26] in experiments with chicks fed on a peanut-meal diet deficient primarily in lysine, observed that the addition of methionine caused a growth retardation that was prevented by a small supplement of lysine. An imbalance involving valine and tryptophan was encountered by Lewis, Elvehjem, and Hart[48] in studies with chicks.

These examples and doubtless others that a thorough review of the literature on amino acid supplementation would bring to light, fit the pattern described earlier; that is, the severity of an amino acid deficiency may be increased by providing in the diet a supplementary amount of an amino acid or a mixture of amino acids other than that which limits growth. In the original example of the niacin-tryptophan-threonine imbalance[31, 32] it was observed that either threonine or a protein such as gelatin, which was deficient in tryptophan, could induce the imbalance. Sauberlich[49] has reported a series of imbalances produced by adding to a diet deficient in a particular amino acid a quantity of a protein that was also deficient in that amino acid (TABLE 10). Under such conditions it appears that an imbalance can be created consistently. There is considerable similarity between this procedure and that used in the rice studies mentioned earlier,[46, 47] in which a more severe imbalance was induced by adding a mixture of all but one of the indispensable amino acids to the diet. Recently, in experiments with rats receiving a low level of fibrin,[50] we have observed a somewhat more complex situation (TABLE 11). In diets containing this protein, some six amino acids are almost equally limiting, and various combinations of them will produce imbalances that are prevented only when all six of them are provided.

TABLE 9

EFFECT OF AMINO ACID SUPPLEMENTS ON GROWTH AND LIVER FAT DEPOSITION OF RATS FED ON RICE DIETS

Diet					
Rice, per cent	L-Lysine HCl, per cent	DL-Threonine, per cent	Essential amino acids	Rate of gain, gm./week	Liver fat, per cent
87	—	—	—	8.5	33.9
87	0.2	0.5	—	22.0	24.0
87	0.4	0.5	—	16.0	10.6
87	0.4	0.5	equal to 3% casein	26.6	13.6
87	0.4	0.5	equal to 3% casein minus: leucine or isoleucine or histidine	8–12	11
87	0.4	0.5	leucine,* isoleucine, and histidine	24.8	15.0

* Equivalent to amount in 3 per cent of casein.

Toward an Explanation of Amino Acid Imbalances

Although certain fairly general criteria can be used to characterize an amino acid imbalance, since the final test of any scientific hypothesis is that it should permit the prediction of analogous events, the characterization cannot be considered complete until amino acid imbalances can be created regularly by satisfying those criteria. In some cases, a supplement of only 0.05 to 0.3 per cent of the second most-limiting amino acid in the diet is sufficient to retard growth. However, it is common knowledge that the growth of animals maintained on diets that provide inadequate quantities of certain amino acids is not regularly retarded when a small supplement of amino acids other than the most limiting one is added to such diets. A more severe change in the amino acid balance, such as that obtained by increasing the levels of all or most of the amino acids except the most limiting one, leads much more regularly to a depression of the growth rate. In such cases it seems quite

TABLE 10

IMBALANCES INDUCED IN RATS BY ADDING NUTRITIONALLY INCOMPLETE PROTEINS TO DIETS MARGINAL IN A SINGLE AMINO ACID[49]

Diet					
Peanut meal, per cent	Oxidized casein, per cent	Corn, per cent	Hemoglobin, per cent	Amino acids	Rate of gain, gm./week
35	—	—	—	—	36
35	20	—	—	—	20
35	20	—	—	0.5% DL-Methionine	30
—	—	75	—	—	9
—	—	75	15	—	1
—	—	75	15	0.55% DL-Isoleucine	21

TABLE 11

EFFECT OF AMINO ACID SUPPLEMENTS ON GROWTH AND LIVER FAT DEPOSITION OF RATS FED ON LOW-PROTEIN DIETS CONTAINING FIBRIN

Diet				
Fibrin, per cent	DL-Methionine, per cent	DL-Phenylalanine, per cent	Amino acid mix	Two-week weight gain, gm.
6	—	—	—	14
6	0.4	—	—	10
6	—	0.6	—	11
6	0.4	0.6		3
6	—	—	Complete*	16
6	0.4	0.6	Complete* minus: leucine, isoleucine, valine, or histidine	3–8
6	0.4	0.6	Complete*	28

* Ingredients: 0.2 per cent L-histidine HCl, 0.2 per cent L-leucine, 0.4 per cent DL-isoleucine, and 0.6 per cent DL-valine.

probable, since the animal must catabolize a fairly large quantity of amino acids that cannot be used for protein synthesis, that the over-all rate of catabolism is increased; consequently, there is some loss of the most limiting amino acid, as was suggested by Salmon.[34] It is difficult, however, to accept the idea that adding as little as 0.05 to 0.3 per cent of an amino acid should produce such an effect. Since the level of tryptophan as well as that of threonine is very critical in creating imbalances in niacin-tryptophan deficient diets,[36] it seems likely that rather subtle differences in the original amino acid balance of the diet may determine how extensive a change will be required to produce an imbalance. The extent of the change required may well depend upon the absolute amount of the limiting amino acid that is provided, a factor that would relate the effect to the rate of growth of the experimental animal.

As to the mechanism by which this effect is exerted, the concept that it is a reflection of increased amino acid catabolism has been mentioned. Sure,[41] Pecora and Hundley,[16] and Block *et al.*[43] observed reduced protein efficiency when an imbalance was induced (TABLE 12), and Sauberlich and Salmon[35] observed some increase in the excretion of the limiting amino acid, but this increase was insufficient to account for the over-all effect. In our own work we have observed that the addition of a quantity of amino acids known to create an imbalance (TABLE 11) will cause an immediate decrease (within 24 hours) in the percentage of absorbed nitrogen retained, in total nitrogen retention, and in food consumption, as shown in TABLE 13.[50] These few observations lead to the conclusion that nitrogen catabolism, probably primarily oxidative breakdown, is increased when an amino acid imbalance is created. A similar wastage of amino acids would be expected if a more severe amino acid deficiency were created directly.

TABLE 12

GROWTH AND NITROGEN EFFICIENCY OF RATS FED ON RICE DIETS SUPPLEMENTED WITH AMINO ACIDS[46]

Diet	Gain, gm.	Nitrogen efficiency,* gm.
Rice	9.2	6.2
Rice + 2.0% DL-lysine HCl	6.6	4.2
Rice + lysine + 0.2% DL-tryptophan	1.8	1.2
Rice + lysine + 1.8% DL-leucine	0.8	0.6
Rice + lysine + 1.0% DL-isoleucine	0.6	0.4
Rice + lysine + 1.2% DL-threonine	28.0	14.4

* Gain per gm. N consumed.

Practical Aspects

There are so many unanswered questions concerning amino acid balance and imbalance that it is difficult to assess the practical significance of this phenomenon. Reports that the requirements for individual amino acids increase with increasing protein intake[7] may bear further investigation. Since a common procedure for demonstrating such effects is to increase the levels of all of the amino acids in the diet except one, the same procedure that is used to create imbalances,[19] it is not improbable that these observations are examples of imbalances in diets that approach adequacy. When the level of dietary protein is greatly excessive, toxic effects may be anticipated.

It is common practice in laboratory experiments to supplement diets with amino acids in order to improve growth, and the beneficial effects of judicious amino acid supplementation are well recognized. It has been during the course of such experiments, however, that most of the examples of amino acid imbalance have been encountered, frequently by inadvertence. Supplementation is generally based on calculation of the percentage of deficit of the various indispensable amino acids, and from this a rough idea of the sequence in which the amino acids become limiting is obtained. In at least three instances we have observed that the calculated sequence is distinctly different from that obtained from growth experiments, and in the case of rice, for example, only the least limiting and the most limiting amino acids occupied the same position by both methods.[17] Inaccuracies in the analytical values for amino acids, inexactness in the estimations of amino acid requirements, and a lack of information concerning the availability of amino acids from proteins can all lead to such errors. Until reliable values for all of these are available, the possibility of unwittingly creating an amino acid imbalance remains.

TABLE 13

EFFECT OF AMINO ACID IMBALANCE ON NITROGEN BALANCE*

	Food intake, gm./day	N intake, mg./day	Urine N, mg./day	Fecal N, mg./day	N absorbed, mg./day	N retained, mg./day	Absorbed N retained, per cent
6% Fibrin (2 weeks)	6.0	65	10.3	5.0	60	50	83
+ Phenylalanine Methionine Histidine (1st day)	3.8	42.8	13.2	4.2	38.6	25.4	66
(2nd day)	3.2	36.8	12.5	2.7	34.1	21.6	64
(3rd day)	5.0	57.5	15.9	2.6	54.9	39.0	71

* See also TABLE 11.

Most of the examples of amino acid imbalance discussed here have been observed in rats. However, since similar results have been obtained in experiments with mice,[19] chicks,[26, 48] dogs,[12] and pigs,[45, 46] it seems likely that all animals are susceptible to such effects. Although no clear-cut cases of amino acid imbalance in man have been reported, Holt[51] has cited an example in which an amino acid supplement caused a growth depression in a human infant, and Hundley *et al.*,[52] working with adult humans, have performed some experiments in which they obtained some evidence for an imbalance and noted considerable individual variation in the response to amino acid supplements. It would thus seem important to investigate thoroughly the significance of amino acid balance and imbalance in nutrition in order to provide a sound basis for decisions regarding amino acid supplementation of foods and feeds.

On the brighter side, we may entertain the possibility that the creation of amino acid imbalances or toxicities will find a therapeutic use in the treatment of virus or neoplastic diseases.[53, 54]

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Discussion

J. B. Allison (*Rutgers University, New Brunswick, N. J.*): Harper has given us a definition for amino acid imbalance and a working hypothesis for the study of the imbalance that is very helpful, indeed. His excellent paper will be very valuable in the correction of certain misunderstandings concerning the concept of imbalance; at the same time, it should stimulate research toward a better understanding of this concept.

I am particularly pleased to note the last sentence in his paper: "On the brighter side, the possibility may be entertained that the creation of amino acid imbalances or toxicities may find a therapeutic use in the treatment of virus or neoplastic diseases." The toxicity of large excesses of methionine is mentioned, a toxicity that is counteracted under certain experimental conditions by increasing the intake of arginine and glycine or by providing glycocyamine. We believe that this relationship between methionine and glycine or glycocyamine at toxic levels reveals an important interrelationship that normally exists and that it may have therapeutic uses under stress conditions. For example, a dog depleted in protein reserves, when fed 0.2 gm. of casein nitrogen/day/kg. of body weight, was repleted very slowly at this low protein intake. After 28 days of repletion the plasma albumin was still low, as in the depleted state. Supplementing the casein diet with a small but optimum amount of methionine increased the rate of formation of plasma albumin. Adding glycine with the methionine improved the synthesis of albumin slightly, but supplementing with an equal mixture of methionine and glycocyamine resulted in a marked rise in plasma albumin even above normal values. A similar rise in serum aldolase was observed to accompany supplementation with a mixture of methionine and glycocyamine. Thus, a mixed supplement of this kind added to the casein diet had a specific effect upon the synthesis of plasma albumin in an animal still markedly depleted in other protein reserves.

Another example of the therapeutic use of this type of supplementation was observed in tumor-bearing rats. A transplanted sarcoma was used for these studies; this sarcoma grew and depleted the animal almost independently of the diet. The sarcoma developed most rapidly in rats fed a 12 per cent casein diet deficient in methionine for optimum growth of the normal tissues. Supplementing the diet with methionine improved the growth of the normal tissues in the presence of the tumor, thereby decreasing the depleting effect of the tumor upon the tissues. Supplementing with methionine plus glycine or, still better, methionine plus glycocyamine, resulted in maximum growth of the normal tissues in the presence of the tumor and made possible a chemotherapeutic approach with the ethylenimines that was otherwise impossible. It should be emphasized that these responses to supplementation of a casein diet by a proper mixture of methionine and glycocyamine are observed only under these conditions of stress, where the animal is depleted in protein reserves.

To return to Harper's concept of amino acid imbalances as it might be applied to the problem of supplementation: one approach would be to establish a reference pattern of limiting essential amino acids. A dietary protein that supplied less than the limiting amounts of one or more essential amino acids in the reference pattern would have a reduced nutritive value, which would be improved by supplementation so that the limiting amino acids would approach the reference ratios. To prevent an imbalance, however, the supplementation would have to be done by increasing the most deficient essential amino acid.

H. E. SAUBERLICH (*Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.*): For some time our laboratory has been engaged in studies on amino acid balance, imbalance, and interrelationships. In general, our findings would corroborate Harper's presentation. Although we recognize the type of amino acid imbalance set forth by Harper, in our investigations we consider amino acid balance and imbalances on a broader scale.

Ample evidence has been presented to demonstrate the occurrence of amino acid imbalances. The occurrence of such imbalances may be quite complex and, at times, not readily understandable. From our experience we feel that imbalances of probably most of the essential amino acids can be produced experimentally. In fact, with certain dietary conditions, relationships to the "nonessential" amino acids may be demonstrated. Imbalances may be produced by use of natural or modified proteins or with individual amino acids. The severity of certain imbalances can be altered to the point of death, while other imbalances may manifest themselves in effects other than growth depression. In certain instances the only apparent effect is in enzyme concentrations or in the accumulation of liver fat. Such effects have been observed for methionine and other amino acids, and this appears to be a general phenomenon associated with an intake of excess calories with relation to the protein level in the diet.

Various factors may influence the balance and imbalance of amino acids in the diet. Such factors include the type of carbohydrate or the level of fat employed in the diet. Several vitamin interrelationships may also influence amino acid balances.[1-3] For instance, niacin may spare tryptophan; pyridoxine may spare methionine; and vitamin B_{12} and folacin may reduce the requirement for methionine. Antibiotics may demonstrate a sparing effect on certain amino acids. Amino acid balance may be influenced also by trypsin inhibitors, treatment of the proteins, and pepsin preparations. Most of these factors would exert an effect only in borderline conditions of amino acid sufficiency in the diet.

In addition, it should be remembered that, although analyses may indicate adequate amounts of amino acids in a diet, the availability or rates of release of the amino acids may have a bearing on the final results obtained. Such observations have been noted, for example, in studies on corn proteins.[4]

The growth depression observed from the feeding of excess amounts of single amino acids in the diet usually has been considered a toxic manifestation. However, from systematic studies on the D- and L- forms of amino acids, we feel that the effects of feeding excess amino acids often may represent an imbalance condition. The growth depression is a peculiarity of each amino acid, and is related to the diet employed. Although the feeding of excessive amounts of amino acids is not a natural condition, such studies may indicate which amino acids are suspect under practical conditions.

The cause of the depression in growth observed when many of the amino acids are fed in excess is not entirely clear. Although food intake is depressed with certain amino acids, this does not always occur. For example, excess leucine may depress growth markedly, but without any apparent reduction in food consumption. The depression does not appear to be necessarily associated with free amino acids in the diet, since certain amino acids fed in excess produce no effect. Similarly, amino acid diets with no protein added may be fed to weanling rats and will permit a growth rate of over 40 gm. per week, comparable to growth obtained with intact protein diets. Likewise, diets containing 80 or 90 per cent of protein may be fed to rats with no resultant depression in growth, yet these same diets will contain amino acids greatly in excess of those fed in the toxicity studies.

When the protein level in the diet is increased, the growth depression caused by the excess amino acid may be reversed. However, there are differences in the effectiveness of the protein employed. In general, the addition of a specific amino acid will not reverse the growth depression.

What is the basic cause of the imbalance phenomenon? In the case of amino acid imbalance studies, the basal diet may actually contain sufficient amounts of the amino acid under study to permit normal growth. Yet, when additional amino acids or certain proteins are added to the diet, that amount of amino acid is no longer sufficient. Why? What becomes of it? Although losses in the urine occur, these losses do not appear to explain completely the cause of the imbalance.[1]

In the case of the tryptophan imbalance, the plasma level of tryptophan was depressed.[1] In methionine imbalances, little depression in the plasma level of methionine occurred.[2] However, in all imbalances studied, the *ratio* of the amino acid under investigation is altered with respect to the other amino acids. The balance of amino acids in the plasma available for protein synthesis may be of high importance. When this balance is altered, passage of amino acids into the cells or enzymatic synthesis and equilibrium may be upset.

Similar effects may occur when excess amino acids are fed in the diet. Usually the amino acid fed in excess occurs free in high amounts in the plasma. Moreover, the level of the other free plasma amino acids may be depressed. Again, this represents alterations in the ratios of the amino acids available for protein synthesis. When increased protein levels are fed in the presence of the excess single-fed amino acid, the levels of the free plasma amino acids are brought into a more normal balance.

For the most part, studies on amino acid imbalances have not been extended to the conditions of practical nutrition. Thus far, it has been necessary to use accentuated conditions to study and demonstrate that imbalances may exist or be created. Such conditions may rarely or never exist under conditions of practical feeding. However, much milder and more subtle conditions may be noted as we look for them and gain additional knowledge of protein nutrition. Evidence of such conditions already exists in practical poultry feeding. As we increase the use of synthetic amino acid supplements and broaden the use and variety of proteins, particularly plant proteins, we may anticipate a greater need to study the balance of the available amino acids in the diet and their relationship to other interacting components.

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ENRICHMENT OF FOODS WITH PROTEIN

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Introduction

The purposes of this paper are threefold: (1) to describe a program of research and development being organized jointly by FAO, WHO, and UNICEF* that has the aim of increasing the production and consumption of high protein foods in underdeveloped areas; (2) to discuss a few of the principles that are of importance in enriching foods with protein; and (3) to mention some of the practical problems involved in increasing the consumption of high protein foods. The focus of this discussion will be on protein malnutrition in technically underdeveloped areas. It is not intended to infer that there are no problems of protein nutrition in advanced countries such as the United States. Previous papers in this symposium have covered this aspect. However, many studies conducted over the past few years have made it abundantly clear that protein malnutrition is a major and still largely unsolved problem in most underdeveloped countries. Indeed, it seems that protein malnutrition is the most important single nutritional deficiency in the world today.

The FAO-WHO-UNICEF Program

There are many types of nutritional deficiency to be found in various parts of the world. However, the most serious of these is protein malnutrition. It would be superfluous to review the voluminous evidence that documents the existence, prevalence, and gravity of protein malnutrition in many areas. However, a few simple statistics may help to point up the magnitude of the problem. Table 1 lists mortality statistics from well-developed countries as compared to a few randomly selected underdeveloped areas. The figures represent a comparison of death rates for all ages and for the selected age groups 0 to 1 and 1 through 4 years. It will be noted that the over-all deathrate is higher in underdeveloped areas. Infant mortality up to 1 year is also strikingly higher in such countries. Percentagewise, however, the difference in mortality in the 1-through-4 age group is tremendous, and this represents the ages at which protein malnutrition is the greatest hazard. Obviously, many factors in addition to malnutrition are responsible for these striking differences. However, competent observers believe that nutritional deficiencies are a large factor, not only as such, but as a predisposing influence in other prominent causes of death, mainly infectious diseases.

Increased production and consumption of milk have been a keystone of the approach of international agencies, as well as many national and local groups, in efforts to combat protein malnutrition. There is no doubt that milk is an effective preventive and therapeutic agent. However, it has become clear that resources in addition to milk are necessary. There are many countries where milk cannot be produced, or where there is little hope in the immediate future of producing it in sufficient quantities. In some places, there are strong local beliefs that cow's milk is not a suitable food for infants and young children. Inadequacies in transport and storage facilities, not to mention economics, further limit the range of practical usefulness of milk in many areas of great need. Many of UNICEF's programs of milk distribution are dependent on surplus dried milk from the United States, a source that cannot be counted on to be available indefinitely. Consequently, efforts have been directed toward finding additional resources of high protein foods that might be useful. Seven criteria have been evolved to select food products that might help to meet this need, namely: (1) they must be already available locally or be capable of local production; (2) they must be within the economic means of the particular population group either to produce or to buy; (3) they must be easily transportable and have a long storage life without refrigeration under conditions of heat and humidity; (4) they must be completely free of any toxic or other deleterious influence; (5) they must have characteristics of taste, odor, or physical properties that will make them acceptable food products; (6) they must have such nutritional values as to be effective protein supplement; and (7) they must be products not already being utilized maximally as human food.

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* FAO is the Food and Agriculture Organization, United Nations; WHO, the World Health Organization, United Nations; UNICEF, the United Nations International Children's Emergency Fund.

TABLE 1*

COMPARISON OF DEATH RATES IN VARIOUS COUNTRIES

Country	Year	Death rates/1000		
		All ages	0–1	1–4
Australia	1953	9.1	23.5	1.6
England-Wales	1953	11.4	27.5	1.2
Sweden	1953	9.7	19.1	1.1
Brazil	1950	13.0	94.1	16.2
Costa Rica	1950	12.2	111.0	15.5
Egypt	1947	21.5	208.4	49.7
El Salvador	1950	14.8	106.4	24.9
Mexico	1950	16.2	138.0	27.8
Thailand	1947	13.4	53.8	17.1

* Reproduced from the *Demographic Yearbook*, United Nations (1955).

On the basis of these criteria, six foods have been selected for study. In their approximate, probable order of potential usefulness, these are: (1) fish flour, (2) soy products, (3) peanut flour, (4) sesame flour, (5) cottonseed flour and (6) coconut protein. Many others could be added to this list, but have been eliminated for the time being, either because their nutritional properties are not well known, because the amount of the raw material is too small on a world basis, or because they cannot be produced economically in the geographic areas of primary interest.

There are a number of reasons for special interest in each of the above products. Fresh fish are available in many of the underdeveloped areas, and available statistics indicate that the production of fresh fish could be expanded very substantially. However, lack of transport and refrigerated storage prevents wider distribution and use of this valuable protein food. The usual methods of preservation such as canning or freezing are too expensive for the groups of greatest nutritional vulnerability. Smoking and drying offer some possibilities, but fish so processed do not have a long storage life under tropical conditions. Recently, several processes have been developed by which either fresh fish, or dehydrated fish meal, can be defatted, deodorized, and finished as an almost tasteless, odorless, nearly white flour. The deodorization step can be omitted for groups that prefer a fishy taste in their foods. These fish flours retain the high biological value of the starting material; they are very stable in storage; and they can be incorporated into bread, into other cereal products, or into soups or stews with excellent acceptability of the resultant food.[1] Since these flours contain 70 to 80 per cent protein, relatively small amounts are needed to supplement children's diets. It is estimated that fish flour can be produced for as little as 12 to 13 cents lb. On this basis a supplement of 10 gm. per day (about 8 gm. of animal protein) would cost only about one fourth of one cent per day per child.

A number of national and commercial groups are now attempting to develop and exploit fish flour. With respect to the international agencies, FAO has encouraged and stimulated the development of suitable processes to produce this substance. UNICEF has entered into an agreement with the government of Chile to install a fish flour plant in that country, and it is expected that this plant will be in operation about September 1957. Fish flour produced by the process to be used in Chile has an excellent biological value, has been found completely safe, and has excellent acceptability.[1] In Morocco, a process is being developed to produce fish flour under commercial auspices. It is estimated that sufficient flour can be produced to supplement the diet of 250,000 school children without interfering with the normal commercial uses of fish for edible purposes.[2] In South Africa, where a fish flour for human consumption has been under development for some time, it is estimated that 300,000 tons of fish can be diverted annually from animal to human use when the program is fully developed.[2] Many other countries are also attempting to increase the consumption of fish and fish products.

Soybean products are of unique interest, since properly prepared soy products have a biological value closer to that of animal proteins than any other commonly used vegetable protein. Furthermore, among the various products to be studied, only soy has been prepared as an acceptable liquid food for infants. TABLE 2, from the work of Bricker, Mitchell, and Kinsman,[3] illustrates the high biological value of soy. It will be seen that soy flour is almost equivalent to milk as judged by its ability to support nitrogen balance in young women. The value of soy in supplementing white wheat flour is also shown. These data apply to adults who have a lower requirement for protein and amino acids than infants or children. However, soy "milk" has been known for many years as a food that is capable of sustaining satisfactory growth in infants and young children. Although there are still some uncertainties as to how completely satisfactory soy milk is for normal young infants, present evidence certainly indicates that it has a biological value unique among the common vegetable proteins. Soy milks are produced commercially in the United States and several other countries. UNICEF, in cooperation with FAO, have been assisting for some time in installing a soy milk plant in Indonesia. This plant, it is expected, will have begun operations in May or June of 1957, and its product will supplement the very limited local production of cow's milk and that which now must be imported.

TABLE 2*

PROTEIN REQUIREMENT FOR YOUNG WOMEN

Protein	Grams protein required per day for 65 kg. subject
Milk	40.0
White flour	69.0
Soy flour	43.3
Soy - white flour†	50.4
Mixed foods‡	46.0

* Reproduced by permission from Bricker, Mitchell, and Kinsman.[3]

† Thirteen per cent soy flour $\simeq$ 36 per cent of nitrogen.

‡ Included egg, ground beef, soy wheat biscuit, vegetables, and fruit (about 55 per cent animal protein).

TABLE 3*

WORLD PRODUCTION† OF OILSEEDS IN 1954

	Metric tons
Total oilseeds‡	54,600,000
Soybeans	19,500,000
Cottonseed	13,700,000
Peanuts	10,600,000
Coconut (copra)	2,850,000
Sesame	1,800,000
Palm kernels	1,000,000

* Reproduced from the *Statistical Yearbook*, United Nations (1955).

† All figures exclude the Union of Soviet Socialist Republics.

‡ Includes soybeans, cottonseed, sesame, rapeseed, linseed, peanuts, and sunflower seeds.

Peanuts, sesame, cottonseed, and coconut are of particular interest since ordinarily they are processed for their oil and the protein-rich press cakes, by-products of the oil production, are used mainly for animal feed or for fertilizer. If it is economic to use these materials as animal feed or fertilizers, it certainly should be economic to use them as human food if carefully controlled processing would produce, in fact, a food suitable for human consumption. TABLE 3 indicates the very large volume of these press cakes produced annually. If even a portion of this production could be diverted to human use, a substantial contribution might be made.

However, relatively little is known about these press-cake proteins as human food, although in one form or another most of them have been used in human dietaries to some extent. There is a large amount of literature on the nutritional values of these press-cake proteins as determined by chemical analyses and animal studies. Unfortunately, many of the data are difficult to interpret, since these proteins are known to suffer nutritional damage if excessive heat is used in processing, and the precise conditions of production are usually not stated. Moreover, there is the ever-present difficulty of translating animal experiments to man.

These and many other factors made it clear that a considersble amount of research and development would be necessary to evaluate fully the usefulness of these high protein foods as supplements to various types of human diets. Fortunately, a grant of funds from the Rockefeller Foundation, New York, N. Y., has made it possible to start the necessary research, and funds are available from UNICEF to procure the food products needed. With these resources, a coordinated plan of research and testing is being developed by FAO, WHO, and UNICEF which, it is hoped, will lead to the increased utilization of these high-protein foods in areas where needed. It should be stated that, even though this program has a world-wide target, there are no illusions that it will alleviate all existing protein malnutrition. It is simply one approach that may make some contribution to an extensive and difficult problem.

Principles

In designing this coordinated plan of research several principles that seem worthy of mention have emerged:

(1) Any food product to be studied must be well identified and reproducible. It must be produced by a process the complete details of which are known. Thus it is hoped that facts accumulated about a product can develop into a systematic body of knowledge that can be interpreted and extrapolated to assist in solving local problems in many different areas of the world without repetition of all of the experimental and developmental work. If the product is standardized, and if successive batches have essentially the same composition and nutritional characteristics, the accumulated basic knowledge about the product should permit application to a variety of problems.

(2) The product must be produced by an open process that is commercially feasible and that can be duplicated in underdeveloped areas. Obviously it is of little value to study a product produced by an elegant laboratory method that could not be used in underdeveloped areas, nor is it of much value to study a food produced by a "secret" process that could not be used freely in the areas of need.

(3) The food must be characterized as to chemical composition and biological value, and it must be shown to be completely safe prior to testing in human subjects. Studies of biological value and safety should be conducted in more than one species of animals except where similar products have already been relatively well studied in these respects.

(4) The food must have such a protein and amino acid content as to make it a useful protein supplement to one or more types of "poor" diets in underdeveloped areas.

(5) This content must be confirmed by actual tests in human subjects. A considerable body of knowledge is accumulating on the protein and amino acid requirements of various age groups, from which it may be possible to predict the value of protein supplements, but there is at this moment no substitute for the direct test. Parenthetically, it might be noted that FAO will publish shortly a summary of available knowledge on predicting protein requirements on the basis of the amino acid requirements of man and the amino acid composition of the foods in question.

(6) The product should offer special possibilities as a practical supplement for infants, preschool children, and mothers, since these are the most vulnerable groups.

(7) The product should be shown to have good storage properties under simple conditions for as long as one year.

The initial objective of the program is to locate at least one source for each type of high protein product and to produce a batch under carefully defined conditions, following the principles enumerated above. This batch would be carefully studied as to composition, biological value and safety in animal studies, and biological value in man. As soon as the true nutritional value and safety of the food was known, assuming its satisfactory values, then it would be made available as widely as possible to research groups in underdeveloped areas and elsewhere for study as to its ability to prevent or cure protein malnutrition when used as a supplement to various types of local diets. In many instances it may be desirable to use combinations of these foods as supplements, depending on local circumstances and the nutritional values of the foods. Should these initial studies indicate the usefulness of a given product or products, additional quantities of essentially the same material could be procured with a reasonable assurance that it would have the same properties as the original batch.

As data and experience accumulate with these foods it should be possible increasingly to predict the solution to other local problems of protein malnutrition, always subject, however, to the practical problems of food habits and preferences.

The next objective might be to establish a facility in the country concerned to manufacture the product for local consumption, a procedure in which UNICEF is interested and is uniquely able to assist. The program has already reached this stage with two of the products, namely, fish flour and soy milk. The success of these two projects will undoubtedly determine whether similar projects will be started elsewhere.

While the initial approach is being built around single, standardized, reproducible products, this, of course, does not rule out the later inclusion of products with improved biological values or with special physical or physiological properties designed to meet special needs. However, knowledge accumulated in the initial studies should speed materially the practical use of these newer products. Indeed, it seems quite likely that the best protein supplements may prove to be, not single protein foods, but combinations of foods The ability of proteins to supplement one another is well known.

With some of the food products it may not be possible initially to procure a product with the desired specifications. This has been true with coconut. However, FAO has received information very recently that a process known as the Hiller process is being used in the Philippines to produce a 20 per cent protein material that is employed to enrich bread and other cereal products. The protein content of coconut press cake is much lower than that of the other press cakes, and relatively large amounts would be required as a protein supplement, even for children. To the best of my knowledge, a practical process to concentrate the protein has not yet been developed. Some difficulties are also being experienced with sesame. For a number of reasons it is desirable to use dehulled sesame seed to produce a low fat sesame flour. A practical commercial method to remove the oil from dehulled sesame seed has not yet been found. A considerable amount of technological and developmental study may be required to produce products with optimal values as human food.

Certain technological difficulties inhibit the use of some of the other products, but these problems do not seem to be insuperable. They center chiefly about the difficulty of producing a press cake with a fat content sufficiently low to give good storage stability without using so much heat that the protein is damaged. This is a particular problem in using screw or hydraulic presses, which are the usual types of equipment already existing in many underdeveloped areas. For economic and other reasons, the first objective in commercial practice is to get the maximum oil yield. To accomplish this, rather high cooking temperatures may be used and these may damage the protein in the press cake.

This, in very brief simplified outline, is the plan as it now exists. There are, however, some other principles and problems encountered in protein supplementation that deserve comment.

Ideally, advantage should be taken of the voluminous data on the feeding of various types of proteins and protein concentrates to animals as a short cut to correcting inadequate human dietaries. However, as mentioned earlier, there are many difficulties and pitfalls in this process. For example, the first limiting amino acid for many proteins in animals is methionine. In man it is more often another amino acid, lysine.[4] In animal nutrition, the nutritional improvement of soybeans by moderate heating has been known for many years. In man, however, there is very little information from which to determine the amount of heat necessary maximally to improve the protein value of soy protein. In growing animals the limiting amino acids in rice seem to be lysine and threonine, both to about an equal degree.[5] Yet in adult man lysine seems to be the only limiting amino acid, and even then only in certain subjects.[6] In growing animals the difference in biological value between white wheat flour and milk is very striking. In adult human subjects the difference is relatively small, as shown in TABLE 2. Most animal experiments are designed to produce a ration on which the greatest amount of lean tissue can be produced at the least cost in dietary protein. This may be a desirable objective in human protein supplementation as well, but it is hardly practicable. Since human beings inevitably vary in what they select to eat, or what they may be fed by their parents, and since young children and adults often eat essentially the same food, it is necessary to design human diets with a considerably greater margin of safety than is needed with growing pigs or chickens, for example. On the other hand, the protein and amino acid requirements of *growing* animals and man do correlate fairly well.[7] Certainly, many lessons and leads can be derived from animal studies but, as emphasized earlier, there is no substitute for the direct test in man.

Another point in comparing experience in animal feeding and human feeding arises from the fact that most animal rations are developed by *substituting* a portion of the protein with another protein that may give a higher protein efficiency and thus permit a lower or less expensive level of protein in the diet. In diets for humans, on the other hand, we usually must be content, from the practical standpoint, with trying to *add* more protein to what they may already be receiving from their customary diet. This brings up a very important question in working with protein supplements in protein-poor diets: If the initial protein intake is low and poor in quality, can protein adequacy be achieved if enough of almost any protein material is added, or must the amino acid pattern be *balanced* to reach protein adequacy? There is much evidence on this point in animal experiments suggesting that, if the protein supplement is very unbalanced in amino acids, then more harm than good may come from the supplement. On the other hand, if the protein is only moderately deficient in a few essential amino acids, then protein adequacy generally can be achieved by simply increasing the intake of protein. In man there is relatively little information on this point, and there is probably no certain, categorical answer. We know that nitrogen equilibrium can be attained in adults with lysine-deficient white wheat flour if the protein intake is increased sufficiently (TABLE 2). We know that peanut flour, which is deficient in both methionine and lysine, can promote nitrogen retention in adults if enough is used, although about 2.2 times as much peanut protein is needed as for animal protein.[8] We know that infants can retain nitrogen and grow rather satisfactorily on soy milk, which is relatively deficient in methionine. However, soy milk provides a considerably higher ratio of protein to calories than human milk, and this may compensate for the relative insufficiency of amino acids.

I wish to offer some evidence that, under certain circumstances, nitrogen retention may be promoted in adults simply by increasing the supply of nonessential amino acids, even with diets very low in protein entirely from vegetable sources. The subject whose data are shown in FIGURE 1 was a normal young man subsisting on a diet adequate in calories (47 cal. kg. of body weight) and other nutrients except for protein, which was supplied at a level of only 26 gm. per day. Of the protein, 70 per cent came from rice (250 gm. per day) and 30 per cent from fruit. There was no animal protein in the diet except about one half gm., which came from butter or margarine. Each of the vertical bars corresponding to one metabolic period consisted of 6 days, so that the entire experimental period depicted in this figure lasted about 2½ months. The figure is a conventional plot of N balance in which the shaded bars above the zero line represent negative balance and the open areas below the zero line represent positive balance. It will be noted that this subject went into marked negative balance initially, came into mild positive balance with certain amino acid supplements (which are not the point of this presentation), and then reverted to zero balance when the supplements were withdrawn. However, your attention is directed to the last two experimental periods, when a mixture of nonessential amino acids was added. You will note the definite and statistically highly significant shift into positive balance. A similar observation was made in a second subject. Under the conditions of our experiments, just as great a response from nonessential amino acids was obtained as from a mixture of the 8 essential amino acids which supplied an equal amount of nitrogen. This work is described more fully elsewhere.[6]

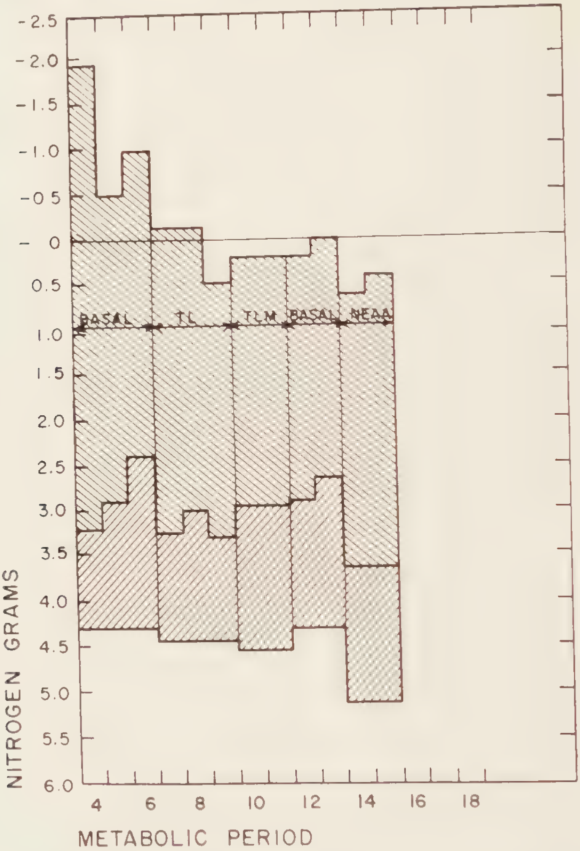

FIGURE 1. Metabolic periods are 6 days each. The data are plotted as average 24-hr. values for each period. Periods 1 to 3 and 16 to 17 (not shown) were on a control 85 gm. protein diet. N intake is plotted from the zero line to the bottom of the shaded area. The bottom shaded area is stool N; the top shaded area is urinary N. Shaded areas above the zero line represent negative N balance; open areas below the zero line represent positive balance.

It is, of course, not new that only a small portion of man's need for nitrogen must be supplied in the form of essential amino acids. This has been shown very clearly by the work of Rose[9] and of many others. However, it was a surprise that a subject subsisting on only 26 gm. of protein from vegetable sources would respond simply to additional nonessential nitrogen. An analysis of the diet as shown in TABLE 4 suggests the reason for this. The rice-fruit diet supplied more than Rose's minimum[10] of all the essential amino acids except methionine and cystine, and possibly lysine. The figures shown in the first column are calculated for total amino acid content of the diet and should be reduced perhaps 10 to 15 per cent to allow for digestibility, which would make lysine borderline in amount. However, since individual subjects show a considerable variation in their requirement for individual essential amino acids, it seems quite possible that this subject was receiving sufficient amounts of all the essentials and therefore had need only for additional nitrogen so that the essentials could be spared for their irreplaceable function.

TABLE 4
AMINO ACID REQUIREMENTS OF MAN VERSUS RICE-FRUIT DIETS
Subject W.M.

Amino acid	250 gm. rice diet* (gm.)	Rose's	
		Minimum (gm.)	Safe (gm.)
Lysine	0.90	0.80	1.60
Threonine	1.00	0.50	1.00
Methionine (+ cystine)	0.98	1.10	2.20
Tryptophan	0.34	0.25	0.50
Phenylalanine (+ tyrosine)	2.61	1.10	2.20
Leucine	2.11	1.10	2.20
Isoleucine	1.26	0.70	1.40
Valine	1.53	0.80	1.60

* These calculations were made from standard literature values for rice and, for the remainder of the diet N, amino acid composition similar to vegetable leaf proteins is assumed.

I do not wish to generalize from these very limited observations except to this extent. If, in a diet as low in protein as the one used in these experiments, the predominant need is simply for more available nitrogen, essential or nonessential, it would seem to follow that "almost any" protein, well balanced or not, would assist in attaining adequate protein nutrition. Obviously, the situation with infants and young children might be quite different because of their higher requirement for protein and amino acids. Additional experimental evidence on this point is badly needed. Not only may the age group make an important difference, but the previous nutritional history of the individuals may also be a critical factor. Subjects who have been depleted of protein are known to retain protein avidly, if given the opportunity, and might react quite differently from subjects with a previously adequate protein intake. A second point is that one cannot consider the possible effectiveness of the protein supplement without considering the adequacy of the rest of the diet. Obviously, little would be gained if the protein deficiency were corrected only to precipitate beriberi, pellagra, or some other deficiency through the increased growth and metabolism permitted by the protein. Nor is it efficient to add a protein supplement to a diet if some other deficiency such as calories, vitamins, or minerals limits the effectiveness of the protein supplement. It is easy to show in animal experiments that the diet must be adequate in all essential nutrients if a protein supplement is to exert its full effect. A simple example is shown in TABLE 5.[11] With the growing rat, a white wheat flour diet was improved not at all by a supplement of vitamins. It was improved only a little by a good protein supplement. However, vitamins and the protein supplement together gave a marked growth response. All of these diets were supplemented with minerals so that protein and vitamins were the only limiting factors.

A third point concerns the criteria to be followed in determining the effectiveness of protein supplements. A number of techniques are available: nitrogen balance in adults, growth in school-age children, the prevention and cure of protein malnutrition, nitrogen balance in young children, growth and nitrogen balance or other measurements in infants are the most commonly used. Each of these has certain virtues and limitations, advantages and disadvantages. All have pitfalls.

TABLE 5
GROWTH OF RATS ON WHEAT FLOUR DIETS WITH VITAMIN AND PROTEIN SUPPLEMENTS

Diet	Supplement	Growth, gm./wk./rat		S.E.
White Wheat Flour	—	1.7	±	0.33
White Wheat Flour	Vitamins	1.6	±	0.15
White Wheat Flour	Six per cent dry skim milk	4.2	±	0.30
White Wheat Flour	Vitamins + 6 per cent dry skim milk	11.3	±	0.50

The rats were weanling National Institutes of Health strain black rats. The growth period was four weeks. Each group contained ten rats. An adequate mineral mixture was added to all diets. The wheat flour contained 1.6 per cent N. The vitamins added were niacin, thiamine, and riboflavin in amounts to simulate commercial enrichment.

Growth measurements in school-age children who receive their supplement through school or other institutional feeding is a very common approach to the problem. In theory, this is simple, since the experiment can be done on a "captive" group and with large numbers of children. In practice the approach has been disappointing. There are examples in the literature where this technique has demonstrated benefit from a particular scheme of feeding. On the other hand, the scientific literature and the unpublished notebooks of many investigators are replete with examples of negative or inconclusive results. This has been true even in underdeveloped areas where protein malnutrition is prevalent, and there has been every reason to expect a measurable benefit from the supplement used.[12] There are probably many explanations for these failures. Certainly malnutrition exists in school-age children and certainly it can be corrected. However, it may be that children suffering from malnutrition are so thinly scattered among children whose growth cannot be improved that the technique is not sufficiently sensitive to detect an effect. Or, as seems quite possible to this observer, by the time children reach school age many of them have their growth pattern fixed, and only a few are capable of responding to improved protein nutrition. Certainly, insofar as protein malnutrition is concerned, we know that this is first and foremost a problem of the preschool-age child. Older children may suffer from inadequate protein nutrition, but surely not to the extent of the younger child. In animals it can be shown that restriction of growth through diet inadequacy during the early growth period leaves a permanent mark. When such animals are given adequate food later in their growth period, they respond with increased growth, but never catch up with their litter mates who received a continuously adequate diet.[13] Data of this kind in children might do much to clear up some of the uncertainties in growth experiments in children.

Among other techniques, nitrogen balance in adults has been widely used, and it certainly provides useful information. However, as others have discussed thoroughly, one cannot equate nitrogen balance and adequate protein nutrition.[14] Furthermore, nitrogen balance in adults does not necessarily indicate what may happen in infants and children who are the primary targets of protein supplements in underdeveloped areas. Nitrogen balance in young children is a valuable technique, but it is notoriously difficult since it requires a degree of voluntary cooperation that is hardly expected from children of this age.

In recent years, studies on the cure of kwashiorkor and prekwashiorkor have been developed to the point where this seems one of the most straightforward and valuable techniques for evaluating protein supplements. Certainly, if a protein supplement has the ability to cure advanced protein deficiency, it seems a reasonable assumption that it would prevent it. There is the opportunity to study the protein, not only during the initiation of cure, but during the maintenance of cure as well. This approach also offers the opportunity to study the supplementary value of a protein directly in the population involved, rather than in the remote recesses of some academic institution.

Growth studies in young infants on formula diets also offer many advantages. While there are certain humanitarian considerations that require careful consideration, such studies are relatively quick and inexpensive. They yield precise information on the limits to the nutritional value of proteins. Since infancy is the time of the highest need for protein and amino acids, these studies also constitute one of the most severe tests to which a protein may be put. Unfortunately, there are only a few centers where such studies are undertaken.

Practical Aspects

The problem of selecting and testing a protein supplement is simple compared to that of getting the protein into established use in a population. Two basic techniques of protein enrichment are available: involuntary and voluntary.

What might be defined as involuntary enrichment is commonly practiced in the United States and other countries. The enrichment of bread with dry milk solids is a well-known example. This technique is effective only where there is a well-developed commercial distribution of a staple food that is a suitable vehicle for the protein. The recipients of the protein supplement generally are unaware of its presence even though it is so stated on the label, and even though there may be much propaganda trying to promote the product on the basis of the enrichment. Nevertheless, the technique is effective since it can be adopted, promoted, and subsidized if necessary to get the protein into people. School or other institutional feeding is another common technique of involuntary enrichment.

Two aspects of involuntary enrichment are commonly overlooked by nutritionists. One is that a protein supplement may be desirable and may find use because of the physical properties it may impart to the product. This is the primary reason that dry skim milk is so widely used in white bread in the United States, even though the nutritionist would like to believe otherwise. It imparts a consistency and texture that the customer likes. Another example is cottonseed flour—one of the products that is being studied in the FAO-WHO-UNICEF program. Insofar as this observer is aware, the only country in the world where cottonseed is widely used as a human food is in the United States, where it is probably needed the least. Again, this protein supplement has found an established use because of the color and physical properties it imparts to certain products not for nutritional reasons. The second aspect of enrichment, one often overlooked, is the problem of formulation. Lysine and methionine are often the shortest amino acids in these protein supplements, and both are rather sensitive to destruction by heat under certain circumstances. Their concentration in the protein may be further reduced by cooking, baking, or other forms of heat, especially in the presence of free sugar. Accordingly, it is not sufficient to know that a protein supplement has the requisite nutritional values; one must also know that it retains these nutritional values in the product of which it becomes an ingredient.

The second technique of protein enrichment is voluntary enrichment. This is far more difficult than involuntary enrichment. The problem is not insurmountable, but the solution may take many forms. Advantage must be taken of local food preferences, habits, and developed tastes and attitudes about food. Ideally, the product might be produced and consumed simply because the people liked it. Practically, this seldom happens, and it never happens quickly. Patient, persistent propaganda and promotion, often aided by economic subsidy, may be necessary until people acquire a taste and develop a demand for the food. Since the complete development of this technique of enrichment invades the fields of agriculture extension, gardening, home economics, sociology, economics, and other esoteric subjects about which this observer knows little, the discussion will be terminated at this point.

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Discussion

Nevin S. Scrimshaw (*Pan American Sanitary Bureau, Regional Office for the Americas of the World Health Organization, and Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.*): Protein malnutrition is now recognized as a major problem for most of the world's so-called underdeveloped areas, which embrace a large part of the earth's surface and its population. WHO, FAO, and UNICEF are all actively trying, within the limits of their resources, to do something definite and helpful about this problem. James M. Hundley has very ably reviewed the principal protein-rich foods that might be made available in sufficient quantity to be of practical value. He has also outlined some of the problems of standardization, biological testing, and commercial production that make the evaluation of these sources relatively slow and painstaking and that require well-trained personnel and laboratory facilities not ordinarily available in the areas where special protein supplements are most needed. The help of the above-mentioned specialized agencies of the United Nations and the grant from the Rockefeller Foundation to the National Research Council to further this work are serving an urgent need.

I also call attention to the principles that Hundley has clearly presented and that must be followed in developing supplementary foods for large-scale feeding to human populations. These principles are essentially those elaborated by the Conference on Human Protein Requirements and Their Fulfillment in Practice held in Princeton, N. J., in June 1955 and sponsored by the Food and Agriculture Organization, the World Health Organization, and the Josiah Macy Jr. Foundation,[1] and later rephrased by the WHO Protein Advisory Committee and accepted by the three international organizations concerned with the problem, WHO, FAO, and UNICEF. The principles in question serve to ensure an orderly and technically sound procedure for developing the proposed protein-rich supplements and to protect both the international organizations and the people of the so-called underdeveloped areas from inadequately tested products. They should be adhered to by all aspiring to contribute to the solution of the world problem of protein malnutrition.

Hundley mentioned the variability of plant products, a factor that plagues all of us who are trying to standardize and test vegetable protein mixtures. A vegetable mixture such as that already presented by Béhar,[2] which contains 50 per cent corn, may vary, not only in the total amount of protein supplied by the corn, but also in the amounts and proportions of the essential amino acids contained in this protein. This is shown graphically in FIGURES 1 and 2. Biochemically speaking, corn is a highly variable food; varieties grown within the same town in Guatemala may vary from 7 to 12 per cent protein, and the protein of the latter variety may also be greatly superior to that of the former.[3] A few years ago, we had no satisfactory way of determining from analytical figures which combination of higher and lower values for the various essential amino acids represented the better net protein value. The concept of a reference amino acid pattern proposed by the Committee on Protein Requirements of FAO[4] and discussed by Allison[5] has been tremendously useful, although it is admittedly still a first approximation; conclusions drawn from it should be confirmed by biological trials. Plotting varieties of corn as in FIGURE 3 makes it possible to estimate the relative importance of the several essential amino acid deficiencies, as well as to compare two varieties of markedly different total protein content. In this case, the higher protein corn has a lower score because it is lower in tryptophan, but the total amount of nitrogen retained per 100 gm. fed[4] should also be higher.

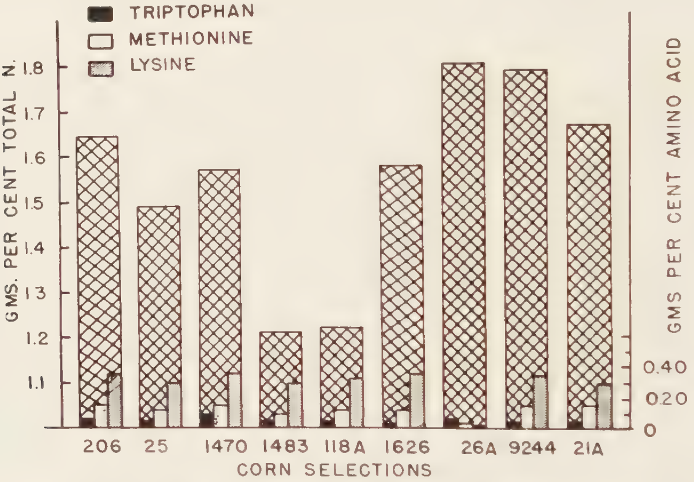

FIGURE 1. Nitrogen and certain amino acids in several Guatemalan yellow corns. Reproduced by permission of INCAP.

TABLE 1 shows some recent results of very considerable interest. Two brothers, 5 and 8 years old, recovering from kwashiorkor, were given the INCAP Vegetable Mixture 8 described by Béhar, not as the sole source of protein, but as 70 and 60 per cent, respectively, of the total protein; the rest of the protein was furnished by corn and beans as ordinarily consumed. After a 5-day period during which nitrogen balance was determined, the protein of

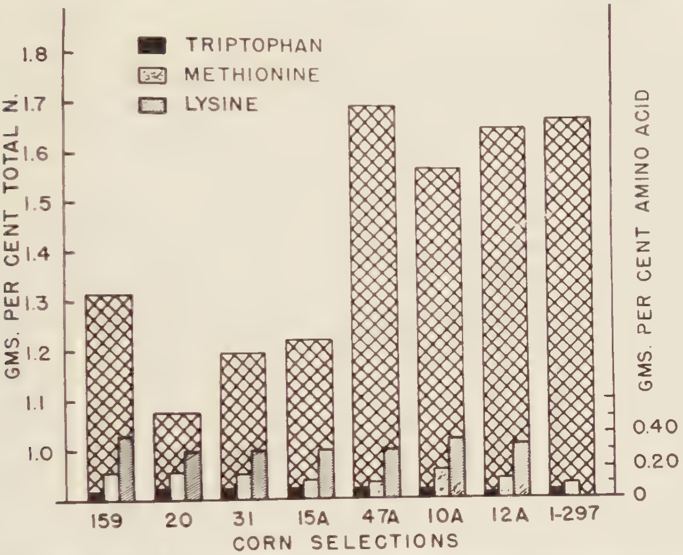

FIGURE 2. Nitrogen and certain amino acids in several Guatemalan white corns. Reproduced by permission of INCAP.

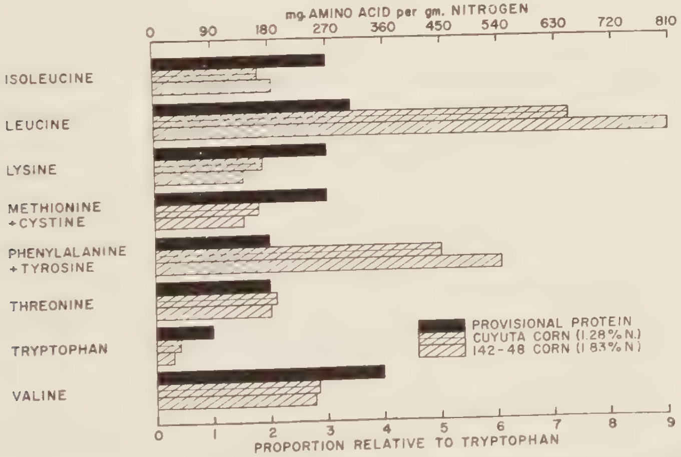

FIGURE 3. Amino acid content of corn compared with reference pattern. Reproduced by permission of INCAP.

of the vegetable mixture was replaced by that of milk, and the nitrogen balance study repeated. In both children the amount of nitrogen absorbed when milk was a major protein source was greater, but the total retained was less. The diet with the vegetable mixture resulted in the retention of 50 per cent more nitrogen in one child and 63 per cent in the other than when milk was given.

Of course these studies must be repeated on additional children, but it appears that 38 per cent of protein from sesame and 12 per cent of protein from cottonseed meal very favorably altered the total value of the protein in a diet in which nearly all of the rest of the protein came from corn and black beans. Perhaps the value of supplementing predominantly vegetable diets, the protein content of which is low in biological value, with other vegetable proteins has been underestimated.

Whether or not there is a place for supplementation with synthetic amino acids can be determined only by further studies. However, Gómez[6] has presented very interesting data showing that nitrogen retention in children is improved when tryptophan and lysine are added to a bean-and-tortilla diet. As György pointed out in his earlier comments, we have recently reported that the addition of methionine in the amount apparently called for by the reference amino acid pattern resulted in a decrease, rather than an increase, in the nitrogen retention of young children on a corn diet already supplemented with tryptophan and lysine.[7] Nevertheless, the addition of these two amino acids, in the proportions suggested by comparison with the reference pattern, had previously resulted in a very significant increase in nitrogen retention, as in the cases presented by Gómez. Our results not only confirm the point made by Harper in his talk[8] and by György in his comments, that amino acid supplementation may result in imbalances that do harm; they also show that, when such imbalances are avoided, striking increases in nitrogen retention can be achieved. Whether this is a feasible method of improving the quality of diets in which the protein is of low biological value remains to be established. It is clear, however, that we should try to learn as much as possible about amino acid imbalances by studying the effects of both food combinations and of the addition of synthetic amino acids to human diets.

TABLE 1

NITROGEN BALANCE COMPARISONS OF MIXED DIETS

	Prot./kg.	Cal./kg.	N Abs., %	N Ret., %	Mg. N, ret./kg.
* Diet with Mix. 8	4.7	132	68	25	189
* Diet with Milk	4.6	130	76	17	126
† Diet with Mix. 8	4.4	106	59	18	122
† Diet with Milk	3.9	104	73	12	75

* 17 kg. male.
† 18.6 kg. male.

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The protein that can be obtained from fresh green leaves is an interesting potential source of protein of good biological value for human nutrition that has not been mentioned in this monograph, and it is one that may some day assume great importance. Leaf protein is known to have a good amino acid balance and, with favorable light, soil, and water conditions, the yields per acre can be enormous. The simple extraction method and the figures presented by N. W. Pirie of England merit serious consideration.[9, 10]

While it is not clear which specific proposals will, in the long run, prove most practical for the solution of the problem of protein malnutrition in so-called underdeveloped areas, a concerted attack with strong international support is being made on the problem. It is evident from the papers and discussions in this publication that substantial progress is being made, and that the next few years will be exciting ones for persons interested in this field.

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J. B. ALLISON (*Rutgers University, New Brunswick, N. J.*): I propose to discuss some results of a study undertaken cooperatively by the Bureau of Biological Research at Rutgers University and a group of laboratories. These results, I believe, will supplement Hundley's very excellent paper.

The investigators conducting this investigation selected the following protein sources for study: whole egg, egg albumin, beef, casein, peanut flour, and wheat gluten. They determined the amino acid pattern in each, and the nutritive values when fed to the mouse, rat, dog, and man. The data obtained emphasized the importance of an adequate daily nitrogen intake to the welfare of the individual and, furthermore, established the fact that the efficiency of utilization of that intake was a function of the pattern of the dietary amino acids. If, for example, 0.4 gm. protein/day/kg. of body weight is required for maintenance in adult man, then that requirement could be met by feeding approximately 0.4 gm. of egg protein/day/kg. If, on the other hand, 0.4 gm. of wheat gluten was fed, only 0.16 gm. would be contributing to tissue synthesis, the remainder being oxidized in energy pathways. Approximately 1 gm. of wheat gluten day kg. of body weight would be needed for maintenance of nitrogen equilibrium. The data obtained, however, indicate that if the biological value is low it is better to improve the pattern of amino acids by supplementation with other dietary proteins than simply to increase the intake to meet the requirements for essential amino acids. Improvement through supplementation is particularly important for growth.

Indeed, the requirements for essential amino acids probably are relatively quite low. Feeding sufficient egg protein to meet the requirement for maintenance supplies at least twice the minimum requirement for amino acids determined through experiments involving feeding of these acids. It may be possible to reduce the amount of egg protein below so-called maintenance requirements if some form of nonessential nitrogen is added to supply the deficit in nitrogen.

It might even be said that the primary need is for nitrogen, with a pattern of essential amino acids that will permit maximum utilization that, in general, the limiting factor is the pattern and not the quantity of essential amino acids in the diet. A reference pattern for evaluating dietary proteins has been sought for some time. The pattern of essential amino acids represented by egg proteins is often used for reference because of the high biological value of these proteins. It is believed, however, that the reference could be developed from various studies of minimum requirements for amino acids, that egg protein might possess some of the essential amino acids in more or less excess. Using such a "luxury" excess might overemphasize some essential amino acid deficiencies in other protein sources. Whether this is true or not, the development of the reference pattern through experiments with mixtures of essential amino acids would give an experimental background to the pattern. The Committee on Protein Requirements of the Food and Agriculture Organization of the United Nations, therefore, studied all the available data on minimum requirements for essential amino acids for maintenance and for growth. This study resulted in the following reference pattern, which Scrimshaw has also described in his excellent paper. This pattern is expressed as mg. amino acid per gm. of nitrogen: isoleucine, 270; leucine, 306; lysine, 270; phenylalanine, 180; tyrosine, 180; sulfur amino acid total, 270; methionine, 144; threonine, 180; tryptophan, 90; and valine, 270. This reference, which will appear in the report of the committee, should not be taken either as final or ideal. It may vary with physiological state, age, and species, but it represents a first step in the determination of minimum requirements for the estimate of nutritive value of dietary proteins.

ECONOMIC ASPECTS OF AMINO ACID SUPPLEMENTATION

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I propose to make a few comments regarding the impact of economics on the question of supplementing foods with amino acids. Economics has a direct bearing on the various aspects of amino acid imbalance discussed by Harper and also on the possible use of amino acids in alleviating the protein nutrition problems described by Hundley and by other contributors to these pages.

First, there is the possibility mentioned by Harper that amino acid supplementation may, under some circumstances, precipitate a vitamin deficiency state. It must be recognized at the outset that any vitamins and minerals that may be needed can today be supplied to the diet at relatively minor cost. Any program of amino acid supplementation should, therefore, have as a necessary accompaniment the provision of those vitamins and minerals known to be present in marginal quantity.

Next, we must consider the economic probabilities of multiple amino acid supplementation; that is, the addition of several essential amino acids at one time. If we must supplement a ton of food with 3 or 4 lb. of the first limiting amino acid, 2 lb. of the next limiting, 1 lb. each of the third and fourth limiting amino acid, and so on, we shall arrive at an economic impasse. The final cost per ton of supplemented food will be impossibly high. In practice, we shall probably be forced to confine amino acid supplementation to meeting only the primary limiting deficiency or, in some cases, where 2 amino acids are almost equally short, to meeting the first and second limiting deficiencies.

Now, to what extent do we supplement to meet the first, or the first and second, limiting deficiencies? Economics tells us we must use the very least amount of supplement that will do a satisfactory job. As Rosenberg[1, 2] and Waddell[3] have recently pointed out, the way to determine this amount is to "titrate" the growth response or nitrogen retention against successive small additions of amino acid. A range of supplementation levels is explored with a number of experimental groups, and the supplement level is varied by very small increments, frequently to be measured in terms of hundredths, rather than tenths, of one per cent of the diet. If the response to the first limiting amino acid is plotted against the logarithm of the percentage of that amino acid in the diet, according to the general method described by Almquist,[4-6] the response reaches a plateau at the point where the first limiting amino acid has come into balance with the second limiting amino acid (FIGURE 1). Further additions of the first limiting amino acid will now have no further effect until, at excessive levels, they may produce a growth depression due to amino acid imbalance. Obviously, economics dictates that no more be used than will bring the response close to the plateau level.

If the attempt is made to remedy the second limiting deficiency, then the levels of both the first and second limiting amino acids must be increased to a point where they are in balance with the third limiting amino acid, as well as with each other. This is obviously a much more complicated problem.

It is apparent that the stringencies of economics will operate to help achieve the end results desired by Harper and others who are concerned about the possibility of producing imbalances by adding amino acids.

My final application of economics to amino acid supplementation is prompted by Hundley's description of an odorless fish flour, containing 70 to 80 per cent protein, that can be purchased in bulk in some places for 12 to 13 cents per pound. This is a phenomenally low price for high-quality protein. In comparison, nonfat dry milk, containing 36 per cent protein, costs about 15 cents per pound in carload lots in the United States. Low-cost fish flour therefore provides us with a rather critical yardstick against which to measure the potential long-term price of lysine.

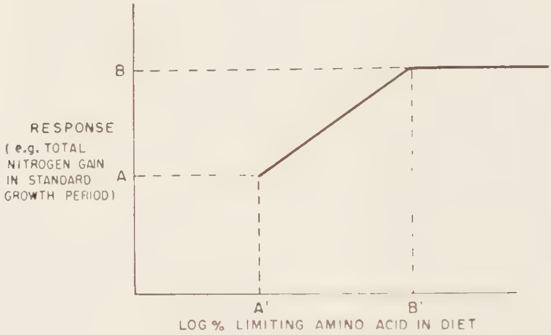

FIGURE 1. Determination of amount of first limiting amino acid required for balance with second limiting amino acid. Point A, A′ represents the response obtained with unsupplemented protein. Point B, B′ is the point of balance. From the difference B′—A′ may be calculated the amount of supplementary amino acid needed to give the optimum balance.

It is not strictly proper to measure a complete protein against a single amino acid, as the one increases the total amount of protein in the diet, while the other merely changes the amino acid balance. However, a comparison is possible if one remembers that in the long run the body can use for its nutrition only units of balanced protein, the remainder of the dietary protein being wasted. Hundley has told us that 10 gm. of fish flour can be added to a diet at a cost of roughly one fourth of one cent per day. This adds 7 to 8 gm. of fish protein. Let us assume that the addition is to a diet providing 50 gm. of protein entirely from wheat. The biological value of wheat protein is generally accepted to be about one half that of meat or milk protein, so that this 50 gm. would have a nutritional value equivalent to about 25 gm. of a balanced protein such as casein. There is evidence from human studies,[7,8] as well as from the animal studies described by Allison in these pages, that wheat protein supplemented with lysine has a protein value similar to that of casein.

Animal feeding studies indicate that a mixture of equal parts animal and wheat protein also has about the same biological value as casein. Let us assume that this observation applies to humans as well. Then, if we add 7 to 8 gm.

of fish protein to our hypothetical diet, we shall balance 7 to 8 gm. of wheat protein, making a total of 14 to 16 gm. of balanced "fish-wheat" protein and leaving 42 to 43 gm. of unbalanced wheat protein. The unbalanced portion of the protein will have a nutritional value equivalent to 21 to 21.5 gm. of casein. In all, the total "balanced protein equivalent" of the diet will be 35.5 to 37 gm. The added cost of 10 gm. of fish flour containing 70 to 80 per cent protein, at 12 to 13 cents per pound, will be 0.264 to 0.286 cents.

How much added L-lysine monohydrochloride would it take to raise the balanced protein value of 50 gm. of wheat protein from 25 gm. to 35.5 to 37 gm.? If we add enough lysine to balance 21 gm. of the 50, then, since the unbalanced protein remaining (29 gm.) will be nutritionally equivalent to 14.5 gm. of balanced protein, we shall have a total "balanced protein equivalent" of 35.5 gm. Similarly, if enough lysine is added to balance 24 gm., the remaining 26 gm. of unbalanced protein will contribute the nutritional equivalent of 13 gm. of balanced protein, and we obtain a total "balanced protein equivalent" of 37 gm. At the generally recommended fortification level of 0.25 per cent L-lysine monohydrochloride by weight of 12 per cent protein flour which, by animal experiments, doubles the protein value of the flour, we shall need 0.437 gm. to balance 21 gm. of wheat protein and 0.50 gm. to balance 24 gm. Equating these quantities of supplement to the cost of 10 gm. of fish flour, we arrive at an equivalent cost for L-lysine monohydrochloride in the range of $2.40 to $3.00 lb. It does not seem too visionary to expect the price to approach at least the upper end of this range, given a market large enough to bring about the full economies of mass production.

These calculations have been carried out with much more precision than our knowledge warrants. They are presented merely to indicate that the cost of amino acid fortification, at least in the case of lysine, is potentially in the range of very low-cost protein additives. Nor is it intended to suggest that amino acid fortification should be regarded as competitive with the use of protein supplements. Ideally, the solution to protein deficiency problems will involve the greatest practicable use of available proteins jointly with amino acid fortification to develop maximum biological value. We should bear in mind, however, that, in view of the great difficulties involved in developing acceptable protein concentrates, amino acid fortification could in many cases bring about an improvement in the nutritional status of protein-deficient populations far more speedily than any other means we can now foresee.

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GENERAL SUMMARY

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It is interesting that no one in these pages has asked the question: What are the absolute protein needs of children? The opinions of contributors who have the task primarily of taking care of sick children and keeping well children well have differed from those of people who are primarily theorists and experimentalists. I think we can say, generally, that the experimentalists are of the opinion that a modest amount of protein is fine for all practical purposes. On the other hand, those whose primary job is dealing with the patient and with the parent tend to believe that a generous amount of protein is a desirable thing in the diet of children in the United States.

Behind some of these differences of opinion is the old question of minimum and optimum intakes. For almost any nutrient we might mention there is most likely a minimum intake necessary for life, an optimum intake compatible with excellent health and, finally, an excessive level of intake that is injurious. It is comparatively easy to define the minimum intake and the level that will cause injury. It is usually very difficult to define an optimum intake, however, because we are not so clear on what constitutes optimum health or how to measure it.

I believe one of the important things in this monograph is the presentation by Stearns and Johnston of some criteria for optimum protein nutrition in children and adolescents. Few parents in this country would dispute the desirability of giving their youngsters sufficient protein to assure them of good musculature in order to help them maintain good posture, to help provide them with a normal childish exuberance and energy, and to help them resist tuberculosis and rheumatic fever. On the other hand, in many parts of the world the question of optimum protein nutrition is academic, while the important problem is simply to meet the minimum; that is, to get enough protein, or protein of sufficiently high quality, into these children to keep them alive. In the more technically developed countries it will be possible to aim at optimum nutrient intakes, but for much of the world we shall probably have to be content if we can achieve minimums.

The second thing of interest to me is the emphasis placed by some of the contributors on individual variability. In this connection I cite the two cases presented in Stuart's paper: one child at the top of the percentiles and the second child at the bottom of the percentiles. Stuart stressed the high degree of variability among individuals observed by his group.

We should note here also that the previous dietary history and stresses to which the child has been exposed can cause large variations in protein requirements. Thus, the intakes of 6 to 8 gm. or more of protein per kilogram of body weight consumed by kwashiorkor patients during recovery are not too high for these unfortunate children, but might be clearly excessive for children whose protein reserves had not been so depleted.

A third point I note is the importance of the amino acid pattern. Protein requirements may be high or low, depending on variables such as those we have just discussed, but whatever they are, it seems always desirable to meet the requirements, insofar as possible, with protein of high biological value, that is, one having an amino acid pattern that permits efficient utilization of protein by the body. The animal proteins are of this type, and it appears possible to "synthesize" such proteins by supplementing low-quality vegetable proteins with certain amino acids, provided we avoid the addition of wasteful excesses.

A fourth thing of interest is the emphasis that a number of the contributors, for example, Talbot, Allison, and Abbott, place on the importance of considering calories in relation to the protein requirement. One could almost say that it is impossible to talk about protein requirement without saying something in the same breath about the calories available.

A fifth point that interests me is Walker's description of the remarkable nature of the Bantu people; also his mention of a problem frequently encountered in technically underdeveloped areas: the tendency of the mother to make the infant feeding mixtures too dilute. I remember, a year ago, visiting one or two primitive localities, not far from Dakar, where milk powder was being distributed. The young men in charge of this distribution said they found it difficult to persuade the mothers to use enough of this powder. The women used only enough to make a chalky looking mixture, and that is what they gave to their children. If only they would have made it more concentrated! It seems to me that in the United States most of us behave in just the opposite way.

A sixth point, made in Hundley's paper and referred to briefly in previous discussions, is the possibility of overcoming protein deficiencies in various parts of the world by using vegetable proteins and mixtures of vegetable proteins with fish flour, various types of beans, peanut flour, and milk, as well as the possibility of increasing the protein quality of the staple food of various areas by the use of crystalline amino acids. The addition of fish flour, vegetable mixtures, and dried skim milk to the native diet, however, creates problems of education, technical "know-how," adequate production, and distribution.

I think many of us whose primary interest is research in nutrition are apt to overlook the fact that the findings of research are not enough. Some means of communication must be found to transmit to people in various parts of the world, including those in the United States, an understanding of the principles and the importance of good nutrition.

About a year ago, in Lagos, Nigeria, I visited a laboratory of physiology where W. S. S. Ladell was carrying out nitrogen-balance studies on 10 or 12 individuals. Ladell was adding peanut flour in various proportions to *gari*, the native dish of Nigeria, which is fermented cassava. Ordinarily this dish makes up 90 per cent of the native diet. Ladell showed that by introducing 10 or 15 per cent peanut flour into this diet he could produce a positive nitrogen balance. He found he could introduce as much as 45 per cent in the fermented cassava and still have a product perfectly acceptable to his 12 subjects.

However, he made this interesting comment: "That is fine. We know that if you add peanut flour to cassava you will have a much better nutrient mixture. But how can you persuade the people to add the peanut flour?"

This is a question of education. In addition, I think, in the case of such supplements as fish flour and soy products, there is not only a tremendous problem of education (by no means do I intend to imply that it is a problem impossible to solve), but there is also a problem of *mañana*, or human inertia. I mention this because about five years ago in Indonesia I was told about the soy milk factory about to be completed there by WHO, FAO, and UNICEF. Since my visit I have maintained correspondence with friends in Indonesia, and this plant, I gather, is still "about to be completed." This tremendous problem of inertia exists in other parts of the world, and in the United States also; and when our hopes for improving the nutrition of a region are based upon the installation of plants intended to provide such nutrients as soy products and fish flour, it becomes a very practical problem.

The last point, and probably the most important, is the concept of the balanced diet or balanced nutrition. This monograph deals primarily with protein nutrition. We have discussed, briefly, balance in protein nutrition. As mentioned earlier, calories, for example, are part of the picture. Although we make one type of nutrient the subject of this publication, we all know that balanced nutrition is the important consideration.

What is balanced nutrition? It is the type of nutrition that supplies a sufficiency, but not an excess, of calories coming from both carbohydrate and fat, and perhaps from unsaturated fatty acids, in adequate ratio with other essential nutrients, including vitamins, minerals, and amino acids. Insofar as I know, the simplest way to achieve balanced nutrition is to have a variety of foods, a variety even within various groups, and not too many calories. However, most of the world cannot enjoy this ideal type of diet and, even in the United States, many people do not take proper advantage of the variety of our foods in order to achieve a balanced diet. So let us keep in mind that a balanced diet is really the nub of this whole problem, and that most of us are trying to find out what a balanced diet really is. More accurately speaking, how can we provide a balanced diet in Lagos or Guatemala, or for the Bantu, as well as for the New Yorker? We must apply our knowledge of nutrition in order to secure diets well balanced in essential nutrients, even when a variety of foods is not available.

Finally, I should like to emphasize once more the importance to the cause of good nutrition of applying the techniques of motivation, communication, and education. What are the most effective and practical ways of translating our knowledge of nutrition into courses of action that will bring about a higher level of health for people in all parts of the world?

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